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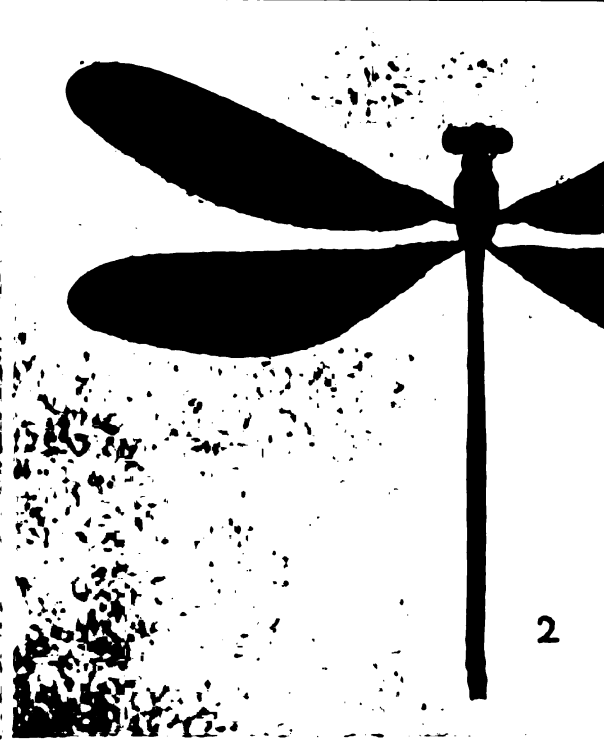
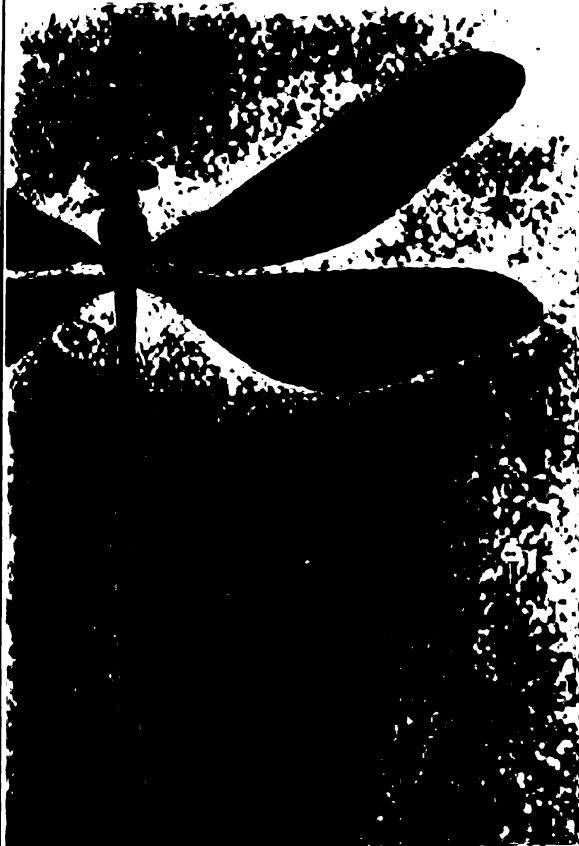
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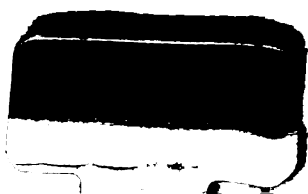
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CONTENTS

1. ALLEN, EZRA, The Cessation of Mitosis in the Central Nervous System of the Albino Rat. (The Journal of Comparative Neurology, Vol. 22, No. 6, December, 1912.)
2. BRUNER, HENRY L., The Mechanism of Pulmonary Respiration in Amphibians with Gill Clefts. (Morpholog. Jahrbuch. 48.)
3. CALVERT, PHILIP P., An Entomologist in Costa Rica. (Annals Entomological Society of America, January, 1913.)
4. CALVERT, PHILIP P., The Fossil Odonate Phenacolestes, with a Discussion of the Venation of the Legion Podagrion Selys. (Proceedings of the Academy of Natural Sciences of Philadelphia, May, 1913.)
5. CALVERT, PHILIP P., The Species of Nehalennia (Odonata) (Entomological News, Vol. XXIV, July, 1913.)
6. CALVERT, PHILIP P., The True Male of Nehalennia integricollis and N. Pallidula, n. sp. (Odon). (Entomological News, Vol. XXIV, October, 1913.)
7. CALVERT, PHILIP P., Obituary, Dr. Horace Jayne. (Entomological News, Vol. XXIV, October, 1913.)
8. CALVERT, PHILIP P., Studies on Costa Rican Odonata. The Waterfall-Dwellers: Thaumtoneura imagos and possible male dimorphism. (Entomological News, Vol. XXV, October, 1914.)
9. CAROTHERS, E. ELEANOR, The Mendelian Ratio in Relation to Certain Orthopteran Chromosomes. (Journal of Morphology, Vol. XXIV, No. 4, December, 1913.)
10. CHAMBERLIN, RALPH V., Two new Otostigmi from India (Myriop). (Entomological News, Vol. XXIV, February, 1913.)
11. CHAMBERLIN, RALPH V., The Lithobiid Genera Nampabius, Garibius, Tidabius, and Sigibius. (Bulletin of the Museum of Comparative Zoology, Harvard College, Vol. LVII, No. 2, November, 1913.)

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13. COLTON, HAROLD S., Results of a Statistical Study of Variation in the Blue Shells of *Pecten Nucleus Irradians* Found at Atlantic City, N. J. (The Nautilus, September, 1914.)
14. JACOBS, MERKEL H., Physiological Studies on Certain Protozoan Parasites of *Diadema Setosum* (Papers from the Marine Biological Laboratory at Tortugas, 1914.)
15. LILLIE, RALPH S., Antagonism Between Salts and Anesthetics. III—Further Observations Showing Parallel Decrease in the Stimulating, Permeability-Increasing, and Toxic Actions of Salt Solutions in the Presence of Anesthetics. (American Journal of Physiology, Vol. XXXI, February, 1913, No. V.)
16. LILLIE, RALPH S., The Formation of Indophenol at the Nuclear and Plasma Membranes of Frogs' Blood Corpuscles and Its Acceleration by Induction Shocks. (Journal of Biological Chemistry, Vol. XV, No. 2, June, 1913.)
17. LILLIE, RALPH S., The Physico-Chemical Conditions of Anesthetic Action. (Science, N. S. Vol. XXXVII, No. 965, June, 1913.)
18. LILLIE, RALPH S., The Physiology of Cell-Division. V. Substitution of Anesthetics for Hypertonic Sea-Water and Cyanide in Artificial Parthenogenesis in Starfish Eggs (Journal of Experimental Zoology, Vol. XV, No. 1, July, 1913.)
19. McCLUNG, CLARENCE E., A Comparative Study of the Chromosomes in Orthopteran Spermatogenesis. (Journal of Morphology, Vol. 25, No. 4, December, 1914.)
20. WILDMAN, EDWARD E., The Spermatogenesis of *Ascaris Megalocephala* with Special Reference to the Two Cytoplasmic Inclusions, the Refractive Body and the Mitochondria: Their Origin, Nature and Role in Fertilization. (Journal of Morphology, Vol. 24, No. 3, September, 1913.)

THE CESSATION OF MITOSIS IN THE CENTRAL NERVOUS SYSTEM OF THE ALBINO RAT

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Biology*

TWENTY-TWO FIGURES

INTRODUCTION

While different observers have recorded the fact that mitosis continues in the central nervous system after birth in mammals which are relatively immature when born, the exact period of its cessation in any one such animal does not seem to have been determined. The purpose of this paper is to record results of studies which I have been pursuing, with many interruptions, for three years, in the effort to discover how long cell division may continue after birth in the central nervous system of the albino rat.

The literature on this phase of vertebrate growth is not extensive. Buchholtz ('90) records mitoses in all parts of the central nervous system of new-born dogs and rabbits and those a few days old. Sclavunos ('99) states that in new-born dogs, cats and white mice dividing cells are to be found in the wall of the central canal of the cord, in its white substance, in the substantia gelatinosa, in the anterior gray horns, at the point of entrance of the dorsal and ventral nerve roots, "among the cells of the spinal ganglia," in the dorsal and ventral septa of the cord, in the dura, and "abundantly" in the arachnoid and under the pia. Hamilton ('01) found mitoses in the cerebrum and spinal cord of the albino rat in animals four days old. Addison ('11), in his study of the Purkinje cells of the same animal, notes mitoses regularly occurring in the cerebellar cortex at twenty-one days, and in one individual at twenty-two days after birth.

MATERIAL AND TECHNIQUE

I am indebted to The Wistar Institute of Anatomy and Biology for the albino rats used in the study and for the use of its laboratory equipment. I am indebted to Dr. Stotsenburg, of the Institute, for a supply of rats as needed and for courteous attention; to Dr. H. D. King for a method of imbedding and staining; to Dr. S. Hatai for valuable suggestions. To Dr. H. H. Donaldson I am especially indebted for constant aid and criticism.

My method of work was in general as follows. Healthy rats of the following ages were used: 1, 4, 6, 7, 12, 15, 18, 20, 30, 52, 70, about 120 days old, and one about two years old. In all, some twenty-five different animals were studied. Record was made of the weight, body-length and sex. After chloroforming the animal, the brain and cord were quickly removed, fixed in Carnoy's fluid and imbedded, the younger specimens in paraffin the older in celloidin and paraffin. Frontal sections were then cut at 8 micra and stained with either iron-alum-haematoxylin or thionin, followed by eosin or erythrosin. Thionin was found to differentiate the mitotic figures with sufficient clearness for this study. By using the mechanical stage, the sections were thoroughly explored with a magnification sufficient to detect mitoses. Only those cells which showed mitotic figures clearly were enumerated; the earlier stages of prophase, being more difficult to identify, were not considered. The Zeiss 4 mm. objective and No. 4 eyepiece usually sufficed; in doubtful cases the 2 mm. oil immersion objective and eye-pieces higher than No. 4 were employed.

Two methods of record were used. With the younger material, diagrams representing the sections studied were outlined and on these the approximate location of the dividing cells was indicated, the number 1 being employed for the cells in section No. 1, the number 2 for those in section No. 2, etc. (figs. 1 to 4). When the examination of a series was complete, this method showed graphically the distribution of the dividing cells in that portion of tissue both in cross section and longitudinally. The other method, usually employed for the older material, was to tally the cells as discovered opposite the number of the section, regional location being indicated by columns if desired.

All the figures are from frontal sections of the cord and brain of the albino rat.

Figs. 1 to 4 Diagrammatic drawings of frontal sections through the cervical cord and at three different levels of the cerebrum of a one-day-old rat. Each figure shows the position of a dividing cell in the section of the series corresponding to that number. The dotted lines outline gray matter. The ventral portion of the tissue appears at the bottom.

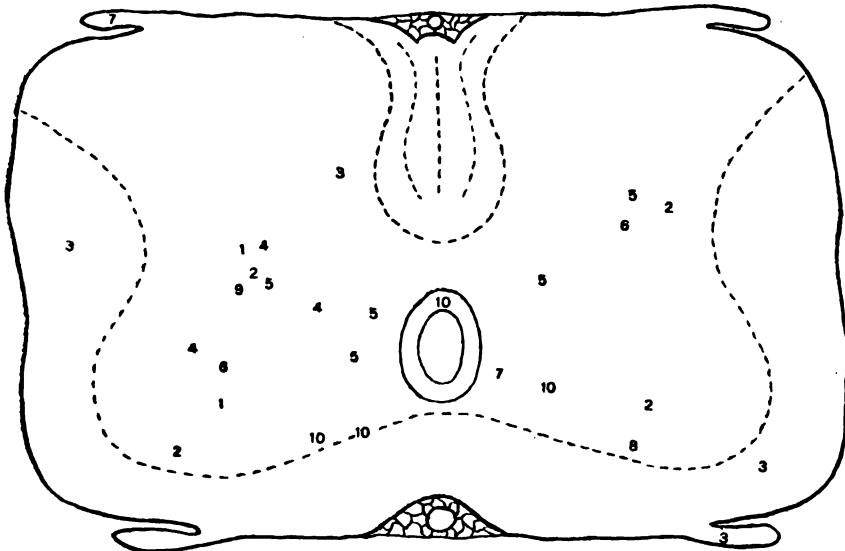
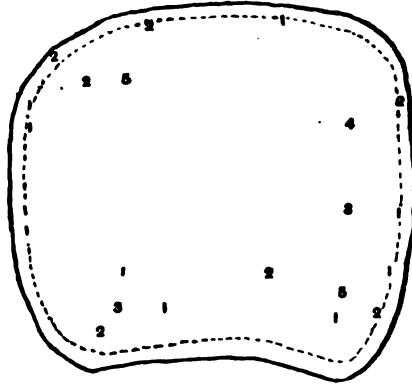
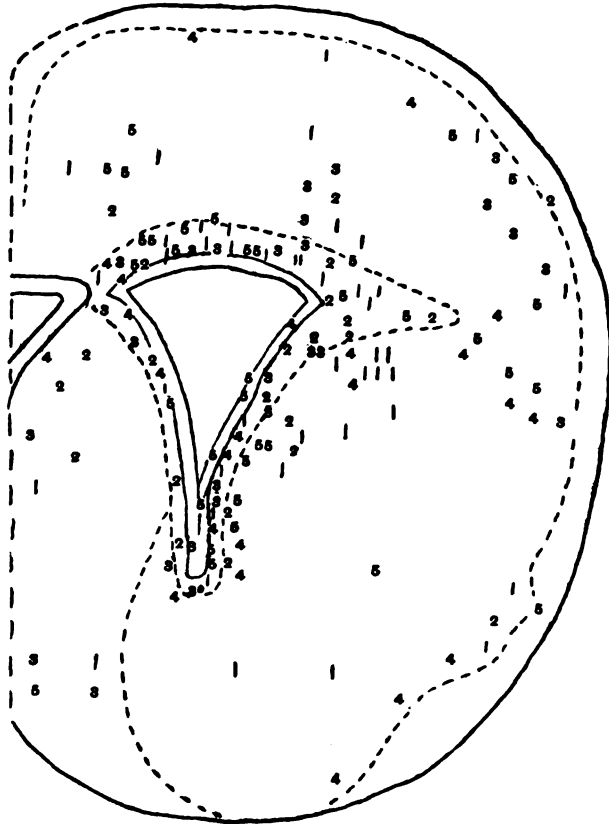


Fig. 1 Distribution of the dividing cells in ten consecutive sections of the cervical cord at the level of its greatest area. $\times 75$.

As to the portions of the central nervous system studied. At first examination was made at various levels throughout the length of the cord, cerebellum and cerebrum to determine whether mitosis was limited to a particular locality. Then, in order to determine numerical relationships, attention was focussed upon certain definite levels. For the spinal cord, the sections showing greatest area in the cervical, thoracic and lumbar levels; for the cerebellum, sections passing through the region showing greatest area, and for the cerebrum sections passing through the optic chiasma were chosen. In each instance frontal sections were used. The number of sections thus studied varied a little—in the cord at least ten and frequently more: in the brain at least five and frequently more.



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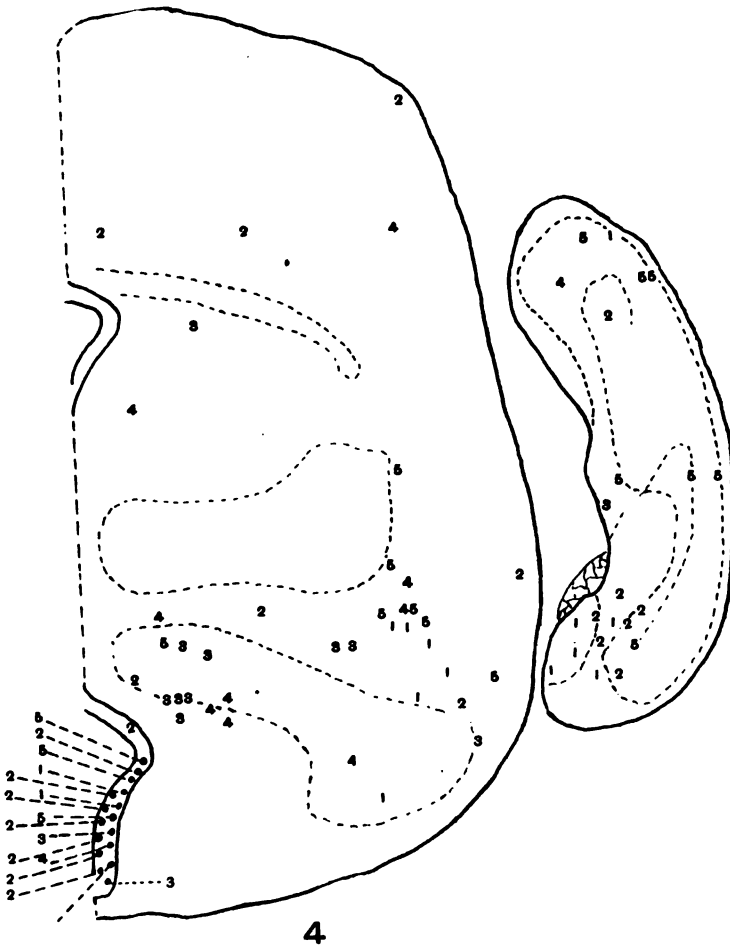


Fig. 2 Distribution of the dividing cells in five consecutive sections cut through the frontal lobe of the cerebrum dorsal to the olfactory lobe. $\times 25$.

Fig. 3 Distribution of the dividing cells in five consecutive sections cut through the optic chiasma. $\times 25$.

Fig. 4 Distribution of dividing cells in five consecutive sections cut through the mid-brain and the occipital lobes of the cerebrum posterior to the lateral ventricles. $\times 25$.

As a basis for determining the comparative rate of cell division the number of mitoses per cubic millimeter was obtained. With the aid of the camera lucida or projection apparatus, drawings were made of the first and last sections of a series. These drawings were then measured by the planimeter and the mean area taken. Since the sections were of a fixed thickness (8 micra) the volume of the tissue in question was readily calculated. The number of dividing cells in the series of sections had already been determined, so that the necessary data for finding the number of dividing cells per cubic millimeter of tissue were at hand.

STATEMENT OF RESULTS

The distribution of mitoses

A brief statement will first be made under this head to be followed by more detailed explanation. The results of this study show that at birth mitoses are occurring in each division of the central nervous system, at all levels of the cord, cerebellum and cerebrum. An examination of figures 1 to 4 will make it evident that the general distribution, however, is unequal. Table 2 (p. 557) indicates at different ages the relative mitotic activity per cubic millimeter at the three principal levels of the cord and at one level of the cerebellum and cerebrum respectively. Figures 2 to 4 show the relative longitudinal distribution at three levels in the cerebrum, as well as the fact that in this organ the region of greatest activity is in the walls of the lateral ventricles. In the cerebellum dividing cells are most abundant in the cortex. Table 2 shows the relative rate of mitosis at different levels for the ages from one day to twenty-five days after birth. This table shows also that cell division stops earliest in the cord, a little later in the cerebellum and still later in the cerebrum. With these data in mind we may pass to the details.

1. The distribution as it appears in frontal section will be better appreciated by a preliminary consideration of the embryonic differentiations of tissue as they appear in the central nervous system. The original epiblastic layer forming the wall of the neural tube is early converted into a protoplasmic framework

(myelospongium) which has a columnar and radial disposition and shows three layers: (a) the innermost or germinal zone; (b) a middle nuclear or mantle zone crowded with daughter cells which have migrated from the actively mitotic germinal layer; and (c) an outer nuclear-free reticular or boundary zone. (His '89 and '04; Hardesty '04; Bryce '08). The seat of mitotic activity is confined at first to the germinal zone but later spreads to the mantle layer (Hamilton '01; Hardesty '04). Activity continues in these two layers with increasing age, but becomes more rapid in the latter (the middle nuclear or mantle). Hamilton ('01), writing of the spinal cord of the albino rat, states: "As

TABLE 1

Giving the percentage of ependymal mitoses in the spinal cord of the albino rat from one day to fifteen days old, based upon the number of mitoses in ten sections from the cervical, thoracic and lumbar levels respectively, excepting that in the fifteen-day rat twenty sections at each level were used.

AGE	TOTAL NUMBER OF MITOSES	EPENDYMAL MITOSES	PERCENTAGE OF EPENDYMAL MITOSES
<i>days</i>			
1	47	4	8.5
4	96	0	0.0
6	115	6	5.2
12	22	2	9.0
15	4	1	25.0
Totals.....	284	13	4.5

The percentage of 13 to 284 is 4.5, the percentage of the ependymal mitoses to the total number of mitoses during the period from 1 day to 15 days.

development proceeds, there is a relative increase of extra-ventricular mitoses, so that by the end of the first day after birth they are greatly in the majority." My preparations from the one-day cord of the same animal confirm this observation. At this age the mitoses are distributed in the ependyma, in the white and gray regions, in the fiber tracts, in the nerve roots, in the enveloping membranes and among the spinal ganglion cells. With advancing age the distribution appears as shown in table 1, the data for which were obtained by adding together the number of mitoses found in ten sections taken from the cervical, thoracic

and lumbar regions of the cord respectively, with one exception. viz., that twenty sections instead of ten were used from each region as a basis for the figures for the fifteen-day specimen.

Hamilton ('01), from observations on twenty-five consecutive sections from the lumbar cord of one new-born rat, concludes that the greatest number of mitoses is in the anterior gray column. This relationship apparently does not persist during the later stages of mitotic activity, as shown by my sections. Furthermore the animals which I have studied show great individual variation at the same age. In addition, the same animal varies greatly in its number of mitoses in series of sections taken at only short distances apart in the same approximate level.

The ependyma of the cerebellum shows very few mitoses in the one-day-old animal. In the cortex they are abundant; there are few in the intermediate tissue. This condition prevails throughout the length of the organ. By the age of twelve days cell division is confined to the cortical area and does not reappear in other portions.

The cerebrum shows a distribution the reverse of that found in the other two divisions. While at birth it is diffuse, the greatest amount of activity is seen in the germinal and mantle layers about the lateral ventricles (fig. 3). The activity external to these layers grows less until at the age of twelve days there is scarcely any cell division elsewhere. Furthermore, the activity about the lateral ventricles is not equally distributed. It is least on the ental lateral surface and the ventral portion of the ectal wall; greatest along the roof and dorsal portion of the ectal lateral wall, where the mantle layer is widest (fig. 7). This distribution is to be seen in the animal one day old at whatever level of the ventricle we may section. In the older animals the region of activity becomes limited to the small portion of the ectal lateral wall lying a little ventrad from the point of union of this wall and the roof. (See further discussion of this zone under the topic Structural Correlations).

2. A comparison of sections from the same rat taken at different levels shows that the rate of mitosis is not equal throughout the length of the central nervous system. The cord is the region

of the least and the cerebellum the seat of the greatest activity, while the cerebrum in that portion where cell division is most rapid exhibits an activity somewhat greater than that of the cord. Table 2 (p. 557) contains figures illustrating these different rates. In addition, mitotic activity is unequal at different levels of the cord and cerebrum. Table 2 brings out this relativity for the cord. The rate of cell division is seen to be lowest in the thoracic and highest on the whole in the cervical portion. In the latter it rises on the sixth day to a rate equal to the most rapid exhibited by the cerebrum (on the fourth day), and more than twice as rapid as that of the cerebrum at six days. From a comparison of numbers of dividing cells at the three levels of the cerebrum, it is found that the greatest number is in the region of sections which pass through the optic chiasma.

This unequal longitudinal distribution is still further illustrated in a small way within the limits of each level, as shown by the markedly greater number of mitoses to be found in two or more consecutive sections of a series, this greater number standing out prominently from the relatively smaller number in the sections immediately preceding and following. Three illustrations follow, chosen from many which show the same relationship. The figures representing the number of mitotic cells in each section of the series run as follows: (1) from the cerebrum—4, 5, 7, 9, 11, 16, 14, 21, 3, 9, 3, 9, 3, 4; and (2) from the cervical cord 4, 0, 2, 3, 5, 8, 1, 4, 2. The numbers italicized in each series mark this grouping of mitotic activity, which may be termed a 'locus' of activity. Similar loci appear in the cerebellum. (3) A brilliant illustration presented itself in the cerebrum of a twenty-five-day specimen, an age when the mantle layer is not so crowded with nuclei as it is in younger material. This illustration will also show the tendency of cells to form groups and to retain a group identity. In this instance three well-marked groups of closely-packed, densely-staining nuclei were found, each well differentiated from the surrounding nuclei, and each showing mitoses. (See mn^1-mn^3 in fig. 10; enlarged view of one group is to be seen in fig. 21). These groups extended through three consecutive sections of 8 micra. I have not endeavored to ascertain

whether such loci are constant in their appearance at fixed points of the system or vary with different individuals. The large number of such loci found within small portions of the tissue at all ages and in each individual examined shows the necessity of using a considerable number of consecutive sections if one desires to make wide application of figures obtained from any one level.

3. Mitosis continues for different periods after birth in the different levels. It ceases first in the cord. Very few dividing cells are to be found in the fifteen-day cord at any level, as shown in table 1 (p. 553). No dividing cells were found in two eighteen-day-old specimens, although sections widely distributed in the different levels respectively were examined. No mitoses were found in the cords of older animals. The eighteen-day period may then be regarded as the time when mitosis has ceased in this portion of the system, having ceased between this and the fifteen-day period.

In the cerebellum it continues until some time between the twenty-second and twenty-fifth day, while in the cerebrum it continues still longer—to a slight degree in material about 120 days old, further discussion of which is taken up under the topics which follow.

4. Table 2 shows the distribution of dividing cells per cubic millimeter at three levels in the spinal cord and at one in the cerebellum and cerebrum respectively. The data for these figures come from consecutive frontal sections at the different levels as fully described in the introduction (p. 552). Each number in the table is not an average from several different rats but is taken from the records of one individual. For a given age, the numbers italicized are from the same animal. It will be noticed that the figures for the cerebellum in each of the animals of the first three ages are not from the same animals as those which furnished the figures for the other levels. This substitution is due to certain technical difficulties which presented themselves, such as failure to get good sections or to obtain true frontal sections in the desired locality. These three cerebella are the only ones of these ages upon which estimations were figured; others might have furnished slightly different figures.

TABLE 2

Showing the number of mitoses per cubic millimeter of nerve tissue in the central nervous system at certain levels. The figures are taken from calculations of the volume of tissue and the number of mitoses in ten consecutive sections at each level of the cord, five in the largest portion of the cerebellum and five in the cerebrum in the region of the optic chiasma (see p. 552). The numbers italicized are from the same individual of that age. The letters (a), (b) and (c) refer to different rats of the same age.

AGE	CORD			CEREBELLUM	CEREBRUM
	Cervical	Thoracic	Lumbar		
<i>days</i>					
1	208	115	259	1597	430
4	437	176	351	2111	447
6	446	236	320	(7-day) 4848	193
12	46	75	14	839	37
20	00	00	00	(a) 00	(a) 18
20	00	00	00	(b) 61	(b) 27
20	00	00	00	(c) 520	
25	00	00	00	00	27

The differences of quantitative distribution which appear from this table if one reads the figures from left to right (according to the same age) have been noted on page 555. If one reads the columns downwards, it appears that the rate of cell division increases after birth (a confirmation of Hamilton '01) until the sixth day in the cord and the seventh in the cerebellum, but in the cerebrum only until the fourth day; after these respective dates the rate decreases rapidly. Cell division has ceased in the cord at the twentieth day, in the cerebellum at the twenty-fifth day, but in the cerebrum is still continuing at this last age at the same rate as for the twentieth day in specimen b.

Since these figures are taken from so limited a number of animals of each age, and from a length of only 80 micra in the cord and 40 micra in the brain at each level, they are to be interpreted as representing the general movement of mitotic activity, not as furnishing the basis for an accurate curve which will show the rate of mitosis after birth. More data must be gathered before such a curve can be constructed. However, the wide difference at each age between the rate in the cerebellum and the other levels indicates that we are safe in concluding that this organ presents the greatest degree of activity. There is good

reason for this condition since at birth the cerebellum is developed to only a slight degree, and its growth thereafter is very rapid during its first twenty-five days of life.

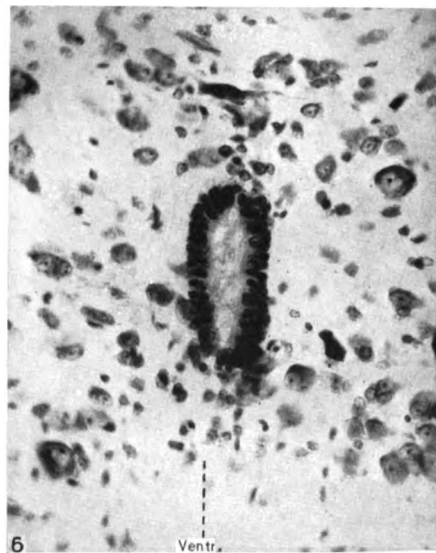
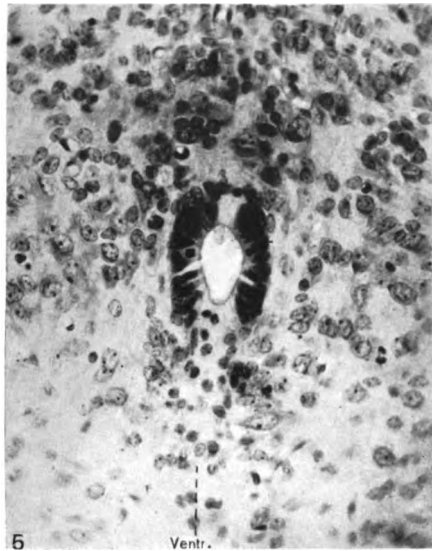
Hamilton ('01) found that the rate of cell division is less at birth than before birth. Unfortunately her paper does not give the embryonic age at which the rate is highest. Otherwise we might fix approximately the two periods in the life history of the albino rat when this factor of growth is most important in the cord and cerebrum. Since the figures given by her are not in terms of number per cubic millimeter, we have not the data needed for an exact comparison of the two phases of growth.

STRUCTURAL CORRELATIONS

1. Correlations in the spinal cord

If we compare sections of the cervical cord from one-day and twenty-day rats respectively, we see several morphological differences (figs. 5 and 6). (1) In the first place, the wall about the central canal of the one-day animal shows germinal cells in mitosis, and shows also that its myelospongium at each end lacks nuclei. Neither of these phenomena appears in the older material. In addition, the form of the canal in the older differs from that of the younger in that it approaches more nearly the strongly elongated oval assumed in the adult cord. (2) The number of cells in the immediate vicinity of the canal of the twenty-day animal is less than in the one-day old. (3) There is greater degree of maturity in all the cells of the older as indicated by their size and cytoplasmic development. And (4) the number of migrating cells is much less in the older than in the younger animal, although the region of greatest migrating activity remains the same in both ages—the region just ventral to the canal.

In most vertebrates the germinal and mantle layers about the canal have been completed some time before birth. One known exception is the chick. Merk ('86) figures the cord of a chick of seven days and five hours old which shows a nuclear-free space in the germinal zone at each end of the canal, a condition found in the albino rat at six days.



Figs. 5 to 11 Untouched photographs of frontal sections through the cord and cerebrum of albino rats of different ages. They show the ventral side downward on the page. The letters indicate the same structures throughout: *ventr*, ventral; *ect*, ectal; *ent*, ental; *r*, roof; *V.iii*, third ventricle.

Fig. 5 One-day rat, cervical cord. $\times 84$.

Fig. 6 Twenty-day rat, cervical cord. $\times 84$.

The germinal layer is completed progressively in the different levels of the cord in the albino rat. The completion is earliest in the thoracic region, where it is fully formed by the twenty-day period. It matures next in the lumbar region, where the wall is closed in nearly every section of the twenty-five-day and in every section of the thirty-day-old animals. In the cervical region, finally, a few sections at the last-named age still show a nuclear-free myelospongium at the ventral portion. The dorsal end is closed at all levels in the interval between the six-day and twelve-day periods.

2. Correlations in the cerebellum

The most interesting feature here is the external granule layer. Mitosis continues in this layer after it has ceased in all other parts of this organ. It ceases when the inward migration of the nuclei has taken place, a movement which is usually completed between the twentieth and twenty-fifth day (Addison '11). I found that in one specimen examined the migration had been completed at twenty days. Addison ('11) found in one of his preparations dividing cells in this layer at twenty-two days. Examination of two twenty-five-day specimens prepared by me revealed no mitoses; neither was any trace of the external granule layer present.

3. Correlations in the cerebrum

The well developed and persistent mantle zone lying along the ectal wall of the lateral ventricles is of particular interest, for in it occur the dividing cells found in the oldest specimens which show mitoses, and this layer, unlike the external granule layer of the cerebellum, never entirely disappears in animals up to two years of age, the oldest which I have examined (fig. 9). The one-day and twenty-five-day specimens both show dividing cells at every level along the entire length of the ventricles. In each case they are most abundant at the level of the optic chiasma. The mantle zone, while in general following the outline of the ventricular wall, is not of the same thickness throughout in any

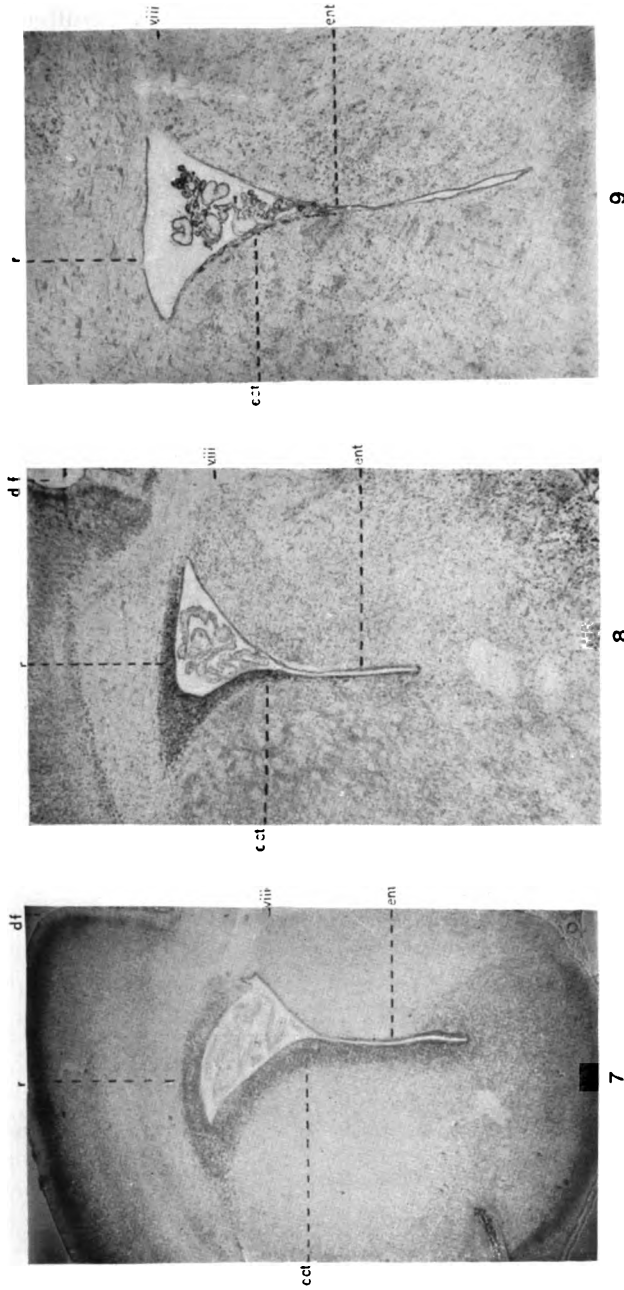


Fig. 7 One-day rat, cerebrum; (one hemisphere) frontal section through optic chiasma to show the condition of the ventricular wall. $\times 21$.

Fig. 8 Six-day cerebrum, as in figure 7. $\times 21$.

Fig. 9 Two-year cerebrum, as in figures 7 and 8. $\times 21$.

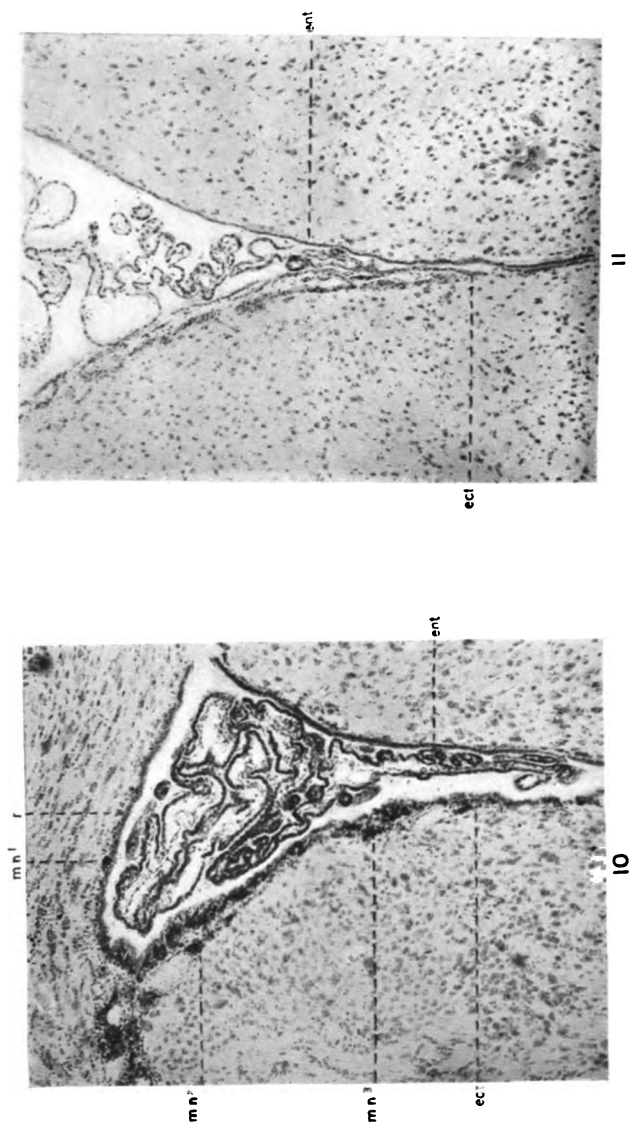


Fig. 10 Twenty-five day cerebrum, dorsal portion of the lateral ventricle to show the mantle zone on a larger scale. $\times 84$. The letters mn^1 , mn^2 and mn^3 refer to loci of active cells (see p. 555).

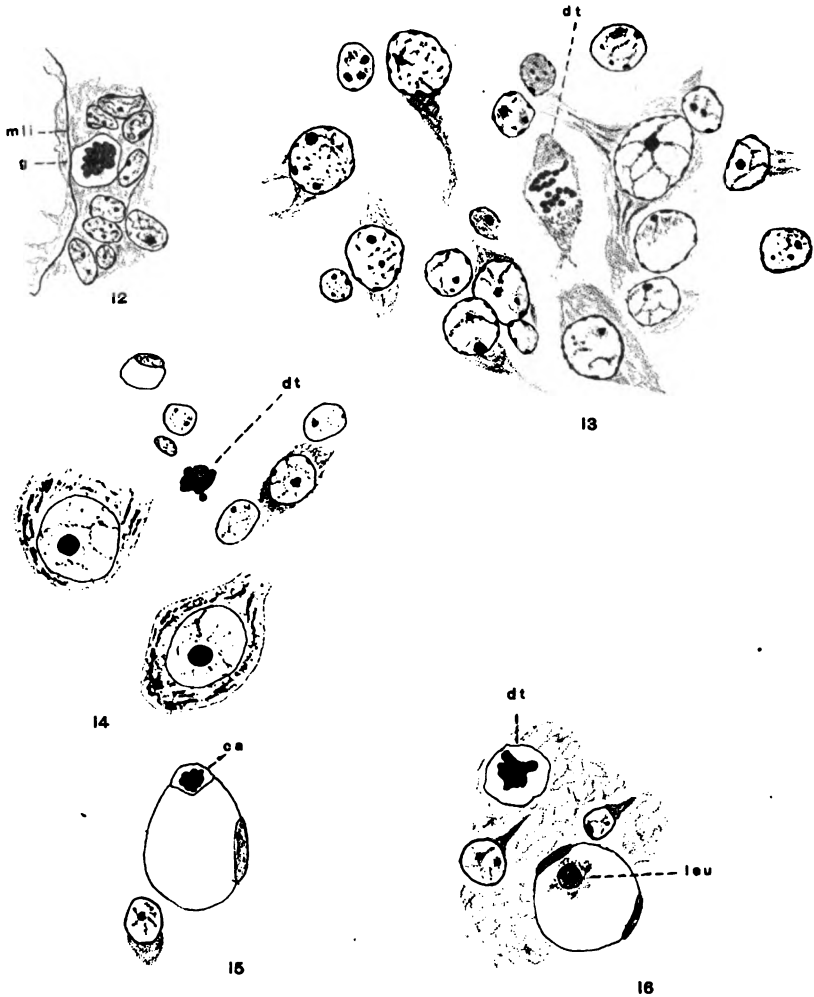
Fig. 11 Two-year cerebrum, as in figure 10. $\times 84$.

frontal section (figs. 7 to 11). It is widest at the point where the ectal lateral wall and the roof meet, and wider all along the ectal lateral wall than along the ental wall. In the one-day animal the layer extends all around the ventricle, but, beginning at the ventral portion, it is gradually reduced in extent until at twenty-five days old it consists of a single layer on the ental lateral wall; on the roof it is in almost the same condition, while on the dorsal portion of the ectal lateral wall and extending well outward at the point it is still several cells in thickness (fig. 10). Reduction is nearly complete along the ental lateral wall at the six-day period (fig. 8). In the twenty-five-day specimens cell division is occurring only in the portion of the ectal lateral wall and roof between mn^1 and mn^3 in figure 10. In this region a total of eight dividing cells was found in a seventy-day animal in five frontal sections at the level of the optic chiasma, these being along the ectal lateral wall, with none on the roof.

The mantle layer persists in this limited region along the ectal lateral wall in the two-year animal, as shown in figures 9 and 11. The active portion of this layer continues *in advanced age* to occupy the region a few cells in width external to the germinal layer, as shown by figures 21 and 22. There is evidently some connection between this layer and the "Uebergangsschichten" of His ('04) shown in his section of the human foetal cerebrum of four months.

NATURE OF THE DIVIDING CELLS

The tissues ultimately found in the nervous system are the neurones, the neuroglia and the connective tissue. In addition we find occasional leucocytes and a relatively greater number of lymphocytes. Hardesty ('04) from his study of the developing neuroglia of the pig, concludes that, "With the present technique there is nothing to show that all the products of the mitoses (germinal cells) in the ependymal layer are not indifferent elements from the first—capable of developing into either neurones or neuroglia." The neuroglia tissue has a double source of origin, arising from both the ectoblast and mesoblast. Hatai ('02) con-



Figs. 12 to 22 Camera lucida drawings of portions of tissue in cord and cerebrum chosen to show dividing cells. All the drawings were made with the Zeiss 2 mm. oil immersion lens and No. 6 eye-piece, tube length 176 mm. The drawings have been uniformly reduced to make the magnification as printed equal 800 diameters. The letters indicate the same structures throughout: *mli*, membrana limitans interna; *g*, dividing cell in germinal layer; *mn*, dividing cell in mantle (middle nuclear) layer; *ep*, ependymal cells; *dt*, cells in differentiated tissue; *leu*, leucocytes; *ca*, cell of capillary wall.

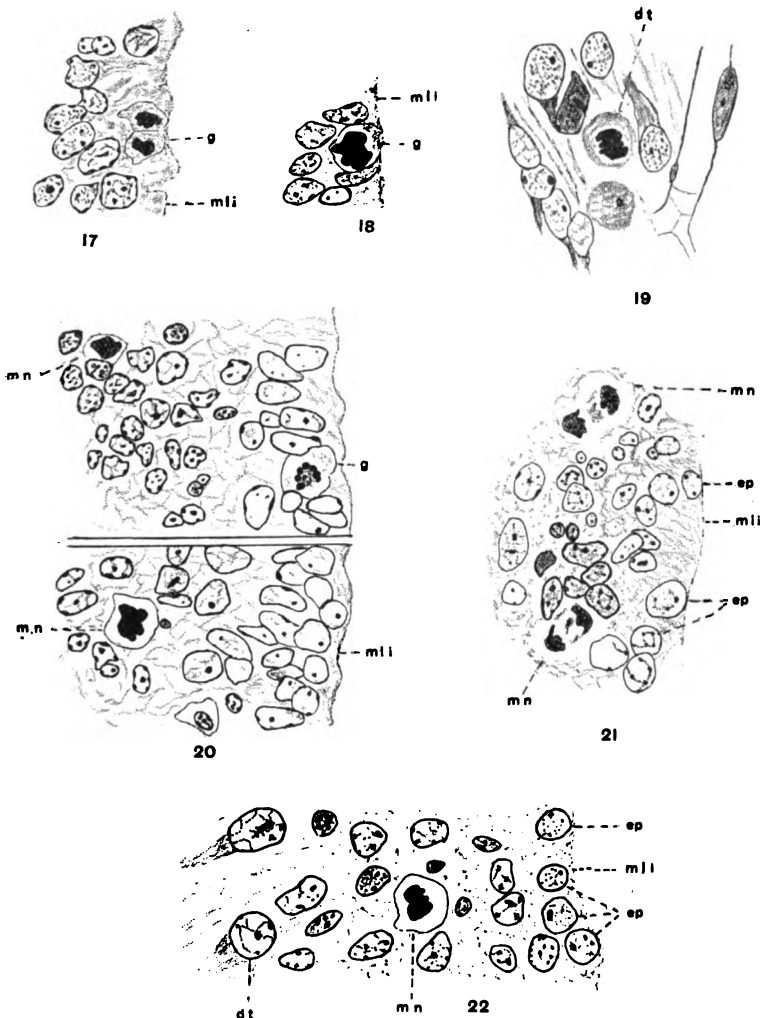
Fig. 12 Four-day rat, cervical cord; dividing cell in germinal layer. $\times 800$.

Fig. 13 Twelve-day thoracic cord; large dividing cell in posterior horn. This cell is one of two dividing cells very near each other, each of which showed its chromosomes in three consecutive sections of 8 micra each. $\times 800$.

Fig. 14 Twelve-day thoracic cord; small dividing cell in posterior horn. $\times 800$.

Fig. 15 Twelve-day thoracic cord; mesodermal dividing cell of capillary found in postero-lateral tract; same section as figure 14. $\times 800$.

Fig. 16 Six-day cerebrum, cortex; leucocyte within a cross section of capillary and a dividing cell. It is difficult to determine whether the granular mass about the nucleus of the leucocyte is its cytoplasm or other material. $\times 800$.



Figures 17 to 22 are from the cerebrum of rats of different ages, showing portions of the zones bordering the ectal lateral wall of the lateral ventricle at about the locality marked *mn*² in figure 10, with the exception of figure 19, which is from the cortex.

Figs. 17 and 18 One-day cerebrum; small portions of germinal layer separated from each other by only a few cells. $\times 800$.

Fig. 19 One-day cerebrum; small portion of the cortical region of the same section as figures 17 and 18. $\times 800$.

Fig. 20 Six-day cerebrum; adjacent portions of germinal and mantle layers. $\times 800$.

Fig. 21 Twenty-five-day cerebrum; two dividing cells in mantle layer, the lower at the edge of a group of cells (*mn*² in fig. 10). (See p. 555.) $\times 800$.

Fig. 22 120-day cerebrum; germinal and mantle layers and two cells showing cytoplasm in the first layer of differentiated tissue. At this age the ependymal cells are clearly differentiated; they began to appear in the twenty-five-day material, as shown in figure 21. The reduction in the number of cells in the two inner layers is to be noticed. $\times 800$.

firms the observations of Fragnito and Capobianco with regard to the mesoblastic source:

From these observations the conclusion is drawn that the neuroglia nuclei in the white rat as well as in the mouse represent two distinctly characterized types: namely, nuclei the structure of which resembles very closely that of the nerve cells, and the nuclei the structure of which resembles very closely that of endothelial cells which form the capillary wall. These two types of nuclei have been derived from the ectoblast and mesoblast respectively. The latter type has probably two sources of origin; that is, they are partly derived from mesoblastic cells immigrating from the meninges (Capobianco and Fragnito), and partly from proliferating endothelial cells of the walls of the capillaries, these cells having separated from the capillary wall and migrated into the surrounding tissue, where they constitute one type of the neuroglia elements.

Hamilton ('01) differentiates two types of dividing cells in the cerebrum and spinal cord, the one, large with cytoplasm well developed and the chromosomes more scattered, the other small without cytoplasm and with the chromosomes solidly bunched. The former, she thinks, develop into nerve cells and the latter into neuroglia. Hardesty ('04) is of the opinion that the neuroglia cells cannot be differentiated from the inward-migrating white fibrous corpuscles. My observations lead me to agree with Hardesty, and so far as my methods of staining reveal any differentiations there seems to be no way of determining whether the small dividing cells are to become neurones, neuroglia cells or white fibrous corpuscles, since the small neurones which show processes very plainly are no larger than some of the dividing cells (figs. 14 and 16).

My preparations indicate that division in the two sizes of mitotic cells does not continue to the same period in the cord and cerebrum. In the cord, the large cells are found dividing in various regions up to the sixth day (fig. 13). While twelve-day specimens still show some mitoses in the germinal zone, the dividing cells of the extra-ependymal regions possess the characteristics of the smaller type. In the cerebrum the chromatic mass in the dividing cell measures up to 7.5×8 micra in the younger material, while in 70-day and 120-day material, a list of measurements runs as follows, the tissue from the two animals having

been prepared in the same manner: 70-day—5 x 4, 5.5 x 5, 6 x 4, 6 x 3 micra; 120 day—6 x 4, 4 x 5, 4 x 4 micra. None were found measuring more than 6 x 4 micra.

The dividing cells which I have enumerated in this study are believed not to be leucocytes or lymphocytes for the following reasons: the size of the nuclei in leucocytes found in blood vessels in my preparations is from 3.2 x 3.2 micra to 3.8 x 3.8 micra, while the chromatin material in the nerve nuclei measures from 4 x 4.2 in anaphase to 7.5 x 8 micra in prophase and metaphase. Moreover, after the dividing cells have disappeared from all other areas they are still to be found in the limited zone along the lateral ventricles of the cerebrum which has already been described. They are to be found also in the neighborhood of capillaries but this association is not constant.

The differentiation from lymphocytes is easier. These are found scattered through the tissue; they are smaller than the leucocytes and are readily recognized by the characteristic nuclei, their chromatin being gathered into one, two or more well separated, densely stained spherical masses, the outlines of which are always smooth and regular, lying in a clear nuclear matrix bounded by a limiting membrane of uniform thickness.

CONCLUSIONS

1. Mitosis ceases in the central nervous system of the albino rat as follows: (a) No mitoses are found in the cord at any level after the eighteenth day. This is somewhat previous to the complete cellular differentiation of the wall of the central canal, a stage accomplished in the thoracic region by the twentieth day after birth, in the lumbar region by the thirtieth day after birth, while at this last-named age the cervical level shows in occasional sections a portion of the wall still incomplete. (b) In the cerebellum mitosis ceases when the migration of the cells in the external granule layer is complete, a condition reached between the twentieth and twenty-fifth day after birth. (c) In the cerebrum mitosis continues with a considerable degree of activity to the twentieth

day after birth, after which it is found to a slight degree in the mantle layer along the ectal lateral wall of the lateral ventricles, this layer persisting at least to the age of two years. The latest observation of dividing cells in this locality was in 120-day material.

2. The rate of mitosis increases for a time after birth, reaching its high point at about the seventh day for the cord and cerebellum and about the fourth day for the cerebrum.

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The Mechanism of Pulmonary Respiration in Amphibians with Gill Clefts.

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With 11 figures in text.

Contents.

	Page
Introduction.	63
Physiological Observations	64
Aquatic respiration	64
Pulmonary respiration	65
The Respiratory Mechanism	68
The closing of the mouth	68
The closing of the gill clefts	69
The closing of the nasal passages	71
The choanal valve of <i>Necturus</i>	72
The choanal valves of <i>Siren</i>	74
The choanal mechanism of <i>Amphiuma</i>	76
Hitherto undescribed muscles in <i>Amphiuma</i>	77
The closing of the choana in <i>Cryptobranchus</i>	79
The choanal valve of larval amphibians	80
Summary and Comment	81
Bibliography	82

Introduction.

CUVIER (1805) accepted TOWNSON'S description of the respiratory mechanism of the frog (1794) and salamander (1795) and advanced the view that the same sort of mechanism exists in amphibians with gill clefts. In reply to the objection that such a mode of respiration would be impossible because the air would escape from the gill clefts, CUVIER (1810, 1836) showed that the axolotl and *Siren* are provided with special muscles for closing the clefts. FISCHER (1864) found similar muscles in *Necturus*, *Amphiuma* and *Cryptobranchus*,

and after a thorough anatomical study of these and allied forms, confirmed CUVIER's view in regard to the general character of the mechanism employed in pulmonary respiration. His conclusions differed from these of CUVIER in one respect, however: he located the apparatus for closing the nasal passage at the choana, whereas CUVIER placed it at the external naris. By the use of modern methods Mrs. WILDER (1909) has shown that the nasal vestibule of *Amphiuma* and *Cryptobranchus* is provided with smooth muscles, apparently homologous with those of the higher urodeles, while ANTON has observed choanal valves in *Necturus*, *Proteus* and *Siren*. Other questions, concerning the nature and function of the choanal valves, concerning the closing of the mouth and gill clefts, also in regard to the sequence of events in pulmonary respiration, have not yet been answered in a satisfactory way.

From the foregoing review it seems desirable that the respiratory mechanism of the lower urodeles should be more carefully studied and compared with that of higher amphibians. Since the lower urodeles resemble in many respects the larvae of higher amphibians, the latter should also be included in the investigation.

This paper is the result of studies carried on during the academic year 1910—1911 at the University of Pennsylvania, where the author held a Fellowship for Research on the George Leib Harrison Foundation.

Physiological Observations.

Acquatic Respiration.

In amphibians with gill clefts the bucco-pharyngeal mechanism of pulmonary respiration is used also in aquatic respiration.

In *Necturus* and the larvae of higher urodeles (*Amblystoma*) regular oscillations of the floor of the mouth occur, elevation being brief and conspicuous while depression is so slow that it may easily be overlooked. During the period of depression the mouth and gill clefts are usually closed and water enters only through the nasal cavity. During the period of elevation water is forced out usually through the gill clefts, sometimes between the lips, not through the nasal cavity.

Siren, *Amphiuma* and *Cryptobranchus alleganiensis* have regular bucco-pharyngeal movements similar to those of *Necturus*, water entering usually through the nostrils and passing out through the

gill clefts. Water may, however, also be discharged through the nasal cavity.

In the frog larva water is taken, under moderate temperatures, both by the open mouth and through the nasal cavity. Under lower temperatures the mouth is closed and water enters only through the nostrils. It is expelled through the gill clefts, not through the nasal cavity.

Pulmonary Respiration.

L. VAILLANT (1863, p. 340) noticed the bubbles of air which escape from the gill clefts of *Siren* after air is taken and concluded that the gills are supplied with atmospheric oxygen in this manner. Sometimes the supply of air, which is taken by the open mouth, may be used only for this purpose, at other times it may be sent directly to the lungs, or a part may be sent to the gills and the remainder to the lungs. In *Cryptobranchus japonicus* B. G. WILDER (1882) observed that the respiratory air is taken through the nostrils, after which bubbles of air escape from the nostrils as the animal sinks in the water. SMITH (1907) gives the following details in regard to the breathing habits of *Cryptobranchus allegheniensis*: 'The air taken in through the nares the instant the snout reaches the surface is probably immediately afterwards mixed with respired air expelled from the lungs; then the greater part of the mixed air is forced back into the lungs by a swallowing movement. The surplus air escapes through the mouth or gill slits as the animal sinks to the bottom.' In *Amphiuma*, according to Mrs. WILDER (1909), air frequently escapes from the gill clefts during the act of inspiration, while in *Necturus* it is often lost through both gill clefts and external nares.

In the following studies I have made use of *Necturus maculosus*, *Siren lacertina*, *Amphiuma tridactylum*, *Cryptobranchus allegheniensis* and larvae of higher amphibians. The respiratory movements of these forms will now be described and a basis will thus be laid for the study of the respiratory mechanism itself.

Necturus. When *Necturus* is immersed in water the regular bucco-pharyngeal movements are interrupted at intervals by pulmonary respiration. As a preparation for this process the floor of the bucco-pharyngeal cavity is strongly elevated in order to remove as much as possible of the respiratory water. This act is followed by the successive stages of pulmonary respiration, corresponding to those

observed by GAUPP (1896, p. 242) in the frog and by myself (BRUNER 1896, p. 400) in the higher urodeles:

1) Aspiration. The jaws are quickly thrust into the air and the mouth is widely opened. At the same time the constrictors of the gill arches probably increase their tonus. 2) Expiration. The escape of air is indicated by the contraction of the abdominal walls. This stage is very brief and is usually complete while the mouth is still open. 3) Inspiration. The entrance of air into the lungs is marked by a wave which runs along the side of the abdomen from its anterior to its posterior end. No air escapes from the nasal cavity, which remains filled with water during the entire pulmonary process. The muscles of the gill arches also prevent the loss of air through the gill clefts. After inspiration the glottis closes in order to retain the air in the lungs. As the animal sinks to the bottom a certain amount of air, probably a surplus remaining in the mouth after inspiration, is forced out either between the lips or through the gill clefts which are opened for the purpose. The removal of this air is evidently a preparation for aquatic respiration, which would be more or less hindered by the presence of air in the mouth and pharynx.

The intervals between respirations in *Necturus* vary according to the temperature, freshness of the water, etc. In running tap water at 56° F. six specimens showed no respirations during ninety minutes. In a small quantity of water which had stood over night at a temperature of about 69° F. the average rate per specimen was nine respirations per hour. In freshly changed water at the same temperature the rate fell to 5.7 respirations per hour.

Siren. Pulmonary respiration in *Siren* is very similar to that of *Necturus*. In the specimens studied, which were about twelve inches long, the air was taken quickly, the animal exposing itself as little as possible above the surface. Under normal conditions no air escapes from the nostrils or gill clefts during inspiration and the nasal cavity is probably filled with water during the entire pulmonary process. The emission of surplus air is not so regular as in *Necturus*, but one or more bubbles may usually be seen, sometimes after a considerable interval.

Amphiuma. The respiratory movements of *Amphiuma* are slow and the various stages of the process are easily distinguished. If the animal is in water the floor of the mouth is strongly elevated as the rostrum rises above the surface and by this means water is

removed from the oral cavity, as in *Necturus*. This preparatory stage is usually followed by two or three respiratory acts, during which the mouth and gill clefts remain closed and air passes in and out through the nasal cavity. The first of these is a normal respiratory act, including aspiration, expiration and inspiration, but the movements of the floor of the mouth do not have great amplitude and relatively little air enters the lungs. In the supplementary acts, which follow in close succession after the first, expiration is usually omitted and aspiration is followed immediately by inspiration, the mouth being well filled with pure air which is sent directly to the lungs. During these stronger inspirations a conspicuous pulse-like wave passes from the anterior to the posterior part of the abdomen.

After the last inspiration the surplus air remaining in the mouth is expelled, usually through the gill clefts, and the animal then resumes its aquatic respiration.

The foregoing account is based on the study of large specimens of *Amphiuma*. An isolated respiratory act which is common in *Necturus* and other amphibians, was occasionally seen in these specimens but two or three inspirations were usually employed to fill the lungs.

Cryptobranchus alleghehiensis. The pulmonary air is renewed in *Cryptobranchus* usually by a single respiratory act, but one or two supplementary acts may sometimes be observed. Air passes through the nasal cavity both in inspiration and expiration and the gill clefts are closed during the entire process. In inspiration the nasal passages are also closed. After the lungs are filled the surplus air is expelled and aquatic respiration is renewed.

Larval amphibians. The statement of VON SIEBOLD (1828), DUGES (1838), and OWEN (1866), that larval amphibians take air by the open mouth and force it into the lungs by a swallowing movement, suggests at once a respiratory process more or less similar to that of *Necturus* and *Siren*. My observations show that such a resemblance actually exists. In the large larva of *Amblystoma tigrinum*, from the moment of taking air by the open mouth to the emission of surplus air through the mouth or gill clefts, the process is essentially the same as in *Necturus*.

In the large larva of the bullfrog, *Rana catesbiana*, the same general characteristics may be easily observed. The precise moment of inspiration and expiration is indicated by movements of the wall of the abdomen and the relation of these movements to the rise and fall of the floor of the mouth can be easily determined. No air

escapes from the nostrils or spiracle during the act of inspiration. The surplus air which remains in the mouth after inspiration is expelled between the lips. Water is evidently retained in the nasal cavity during the entire pulmonary process.

The Respiratory Mechanism.

The preceding observations show that the respiratory movements of amphibians with gill clefts are very similar to those of higher amphibians. They also indicate the existence of a very definite mechanism for the control of the respiratory media, although evidence of more or less variation has been found in different forms. In the following description of this mechanism special attention is given to parts hitherto unobserved and to those whose connection with the respiratory process has not been emphasized. I include here, especially, the arrangements for closing the mouth, gill clefts and nasal openings. The more conspicuous muscles of the mouth and pharynx have been described.

The Closing of the Mouth.

When the mouth is filled with air or water the elevation of the floor of the cavity tends to separate the jaws. This is chiefly prevented by a tonic condition of the adductor muscles of the mandible and by close contact of the lips (see Fig. 3). If the adductor muscles are cut on one side of the head, bubbles of air escape from the mouth on the operated side, especially during the stronger inspirations. Similar losses were observed after the upper and lower lips were divided on one side at a point midway between the rostrum and the angle of the mouth, the adductor muscles being in a normal condition. In all of the urodeles studied the lips have the same general form and they probably have the same function in connection with respiration.

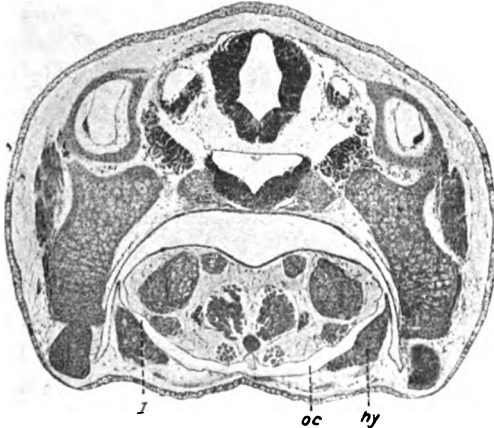
In *Necturus* and *Siren* the retraction of the lips seems also to be an important factor in closing the mouth. Electrical stimulation of the masseter muscle causes both adduction of the mandible and retraction of the two lips and the same effect undoubtedly follows the contraction of the muscle under natural conditions. In *Necturus* this is apparently due to a close union of the sheath of the muscle with the connective tissue of the lips at the angle of the mouth. In *Siren* a bundle of fibres from the masseter is inserted at this point.

Finally I must mention a small muscle, the submentalis of *Cryptobranchus*, which aids in closing the mouth in this form. FISCHER (1864, p. 47) states that this muscle prevents the outward rotation of the lower margin of the mandible, but he is probably in error when he adds that such a rotation would otherwise follow from the contraction of the masseter muscle. A rotation of the mandible, such as FISCHER mentions, is easily produced by depression of the anterior median portion of the mandible, and since the two halves of the mandible are joined at the rostrum only by soft tissues, such a depression would be very likely to occur during inspiration if it were not prevented by contraction of the submentalis muscle. By such action, the submentalis prevents the separation of the jaws and thus assists in the act of inspiration.

The Closing of the Gill Clefts.

During branchial respiration the gill clefts are closed in order to prevent the entrance of water from the outside when the floor of the mouth is depressed. During pulmonary respiration they are

Fig. 1.



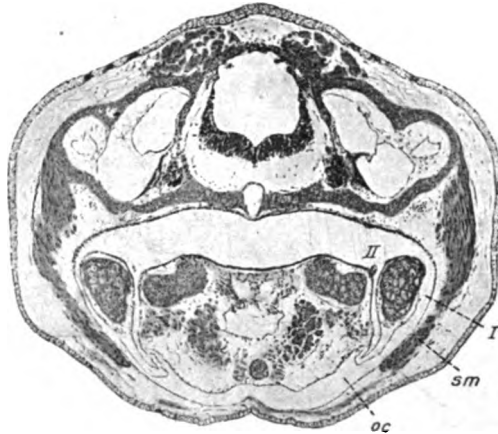
Triton alpestris. Transverse section of an 18 mm. larva. *hy* hyoid arch, with valve-like fold above; *I* first gill cleft; *oc* opercular chamber.

closed to prevent the escape of air from the pharynx and the entrance of water from the outside. The closing of the clefts is due primarily to the action of the constrictor muscles of the gill arches, but these may be assisted by the posterior part of the mylohyoid. The former were long ago observed by CUVIER (1810), MARTIN-SAINT-ANGE (1831)

and FISCHER (1864); the participation of the mylohyoid in closing the gill openings was pointed out by FISCHER.

Accessory structures which aid in closing and protecting the gill clefts are the soft rakers at their inner margins and certain folds or membranes on the outside. The simplest conditions, though probably not the most primitive, occur in the lower urodeles, in which the gill clefts open directly to the exterior and not into an opercular chamber. In *Cryptobranchus* and *Amphiuma* the single cleft on each side of the neck is guarded by two fleshy lips, one behind and one

Fig. 2.



Triton alpestris. Transverse section of an 18 mm. larva. I and II gill clefts, the second closed below by the united branchial membranes; oc opercular chamber; sm posterior part of submaxillaris muscle in the opercular membrane.

in front of the opening. Gill rakers are entirely wanting in *Amphiuma*; in *Cryptobranchus* they are reduced to rudimentary papillae which are more or less irregular in their arrangement. In *Necturus* the two gill clefts on each side are surrounded at their inner margins by short blunt papillae, from six to twelve in number, and so arranged that those on one side of the opening alternate with those on the other side. Externally a fleshy lip protects the anterior margin of the first cleft and a similar fold lies behind the posterior cleft. A single fold between the two clefts furnishes protection to both. The three gill clefts of *Siren* are provided with conspicuous gill rakers similar in form to those of certain fishes, but composed entirely of soft tissues. The external folds are similar to those of *Necturus*,

but the adjoining margins of the second and third clefts are furnished with separate folds.

In the larval salamanders accessory structures for closing and protecting the gill clefts are strongly developed (Figs. 1 and 2). In all of the forms examined, including *Triton alpestris*, *Salamandra maculosa*, *Salamandrina perspicillata* and *Amblystoma tigrinum*, I find essentially the same conditions. All of the clefts, excepting the first, or hyobranchial, are protected internally by conspicuous gill rakers, similar in form and structure to those of *Siren*. The dorsal half of the first cleft is protected only by the operculum; the ventral half is guarded, in addition, by a membranous fold which is attached to the inner surface of the hyoid arch. Externally all gill clefts open into an opercular chamber and are protected by an opercular membrane (gular fold), which arises in front of the first cleft. The muscle of the operculum acts as a constrictor of the pharynx and assists in closing the clefts. The operculum is followed by a series of smaller membranes which are attached, one to each of the branchial arches. By the union of adjacent membranes below the clefts, the latter are completely surrounded except at their dorsal angles, where the membranes terminate at the base of the gills. Along the line of attachment to the branchial arches the membranes have a considerable thickness which may equal that of the arches themselves, but they become thin toward their free edges which lie near the posterior margin of the operculum. These membranes are somewhat elastic structures which follow the movements of the gill arches, diverging from each other when the clefts are open and overlapping more or less closely when the clefts are closed. In the latter position the contact of their thickened bases makes the closing of the clefts secure. That the closing of the clefts is effective during the act of pulmonary respiration has been repeatedly demonstrated by observations on the large larva of *Amblystoma tigrinum*.

In the frog larva the gill clefts can not be wholly closed but the escape of respiratory air can be prevented by other means. According to SCHULZE (1892) the posterior part of the peribranchial chamber can be closed by two muscles, the subbranchialis and diaphragmatico-praecordialis.

The Closing of the Nasal Passages.

In *Amphiuma* and *Cryptobranchus alleganiensis* the external naris leads into a short vestibule whose inner portion can be closed and opened by a smooth muscle mechanism (Mrs. WILDER, 1909).

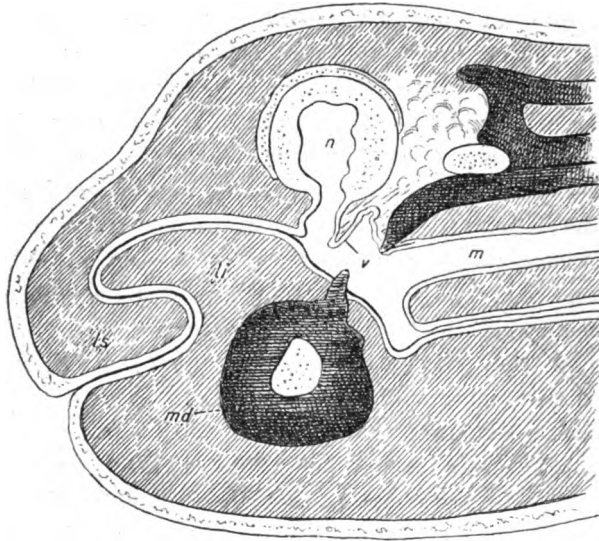
An apparatus for closing the choana is also present in all of the lower urodeles examined (*Necturus*, *Proteus*, *Siren*, *Amphiuma* and *Cryptobranchus*) and also in the larvae of higher amphibians.

The different forms of this choanal mechanism must now be described.

The Choanal Valve of *Necturus*.

The internal nasal opening of *Necturus* is a narrow slit which lies near the angle of the mouth, between the palato-pterygoid and

Fig. 3.



Necturus maculosus. Transverse section through choana. *ls* upper lip; *li* lower lip; *m* oral cavity; *n* nasal cavity; *v* choanal valve, on the median side of the opening, from which a short postnasal canal leads to the cavity of the mouth.

vomer, on one hand, and the antorbital cartilage, on the other. When the mouth is closed the mandible lies directly below the choana, but a free passage is left between the opening and the medial part of the oral cavity. This postnasal passage is formed, in part by a depression between the vomer and the palato-pterygoid, in part by a notch in the dorsal margin of the mandible between the dental and splenial bones. From the roof of this passage, on the median side of the choana, hangs the valve which FISCHER (1864, p. 114) and ANTON (1911, p. 182) have briefly described. The valve is a

triangular fold of mucous membrane (Figs. 3, 4, and 5) which is attached to the median and posterior margins of the opening. Its anterior angle extends forward a short distance in front of the opening and inserts in the connective tissue behind the lateral portion of the premaxillary bone. The valve is not provided with muscles

Fig. 4.

Fig. 5.

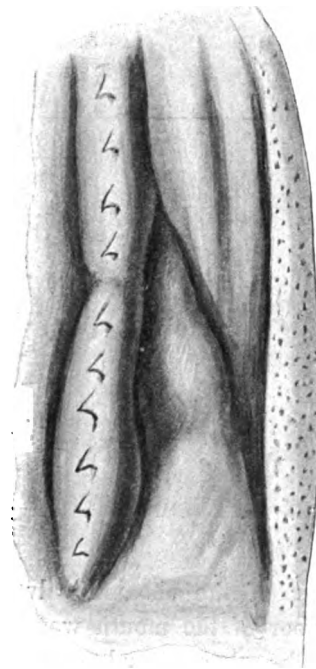
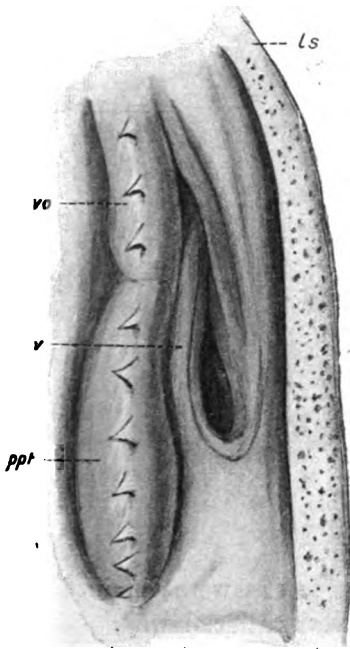


Fig. 4. *Necturus maculosus*. Choanal region, with the choanal valve open. The anterior end of the valve is inserted under a fold of mucous membrane which is pushed aside to show the attachment. *ls* upper lip; *ppt* palato-ptyergoid; *v* choanal valve; *so* vomer.

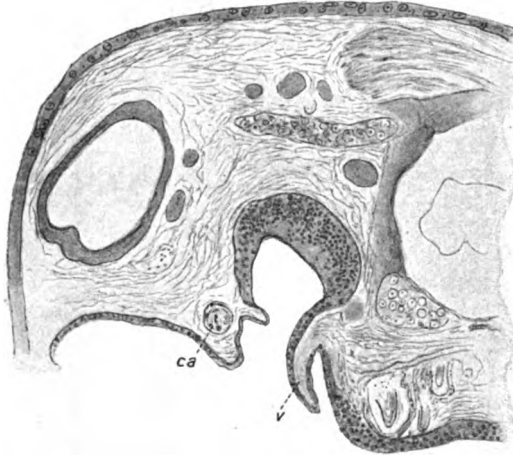
Fig. 5. *Necturus maculosus*. Choanal valve closed.

and acts in a purely mechanical way, being opened by pressure of the respiratory medium in the nasal cavity, while it is closed by any movement of the respiratory medium through the postnasal passage toward the choana.

The function of the choanal valve of *Necturus* has been tested by cutting away the entire left valve in a large specimen, which was then placed in a shallow dish and covered with water. The normal respiratory movements, both branchial and pulmonary, were promptly resumed. At each pulmonary respiration a certain amount

of water was first forced out of the nostril on the operated side and this was followed by a succession of air bubbles. No air and little, if any, water was lost on the right side. During branchial respiration a strong current of water streamed from the left nostril when the floor of the mouth was elevated; little or none escaped from the right opening — the losses being easily accounted for by the mere

Fig. 6.



Siren lacertina. Transverse section through the choana. *ca* antorbital cartilage; *v* median choanal valve.

closing of the choanal valve. Similar results were obtained when the floor of the mouth was elevated in an artificial way.

The choanal valve of *Proteus* is similar to that of *Necturus*, as stated by ANTON (1911).

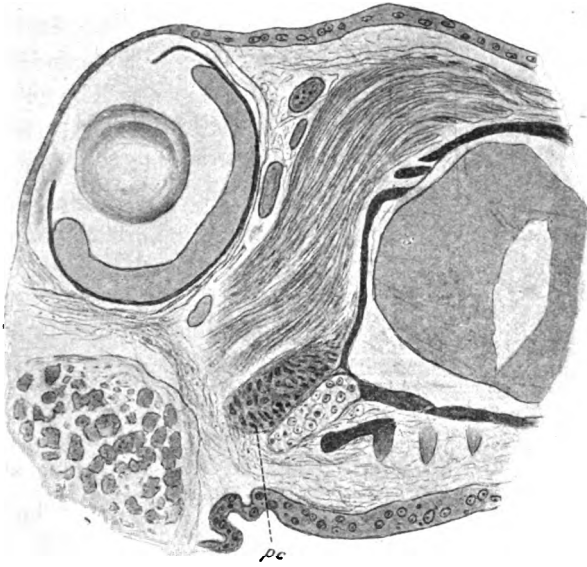
The Choanal Valves of *Siren*.

The inner nasal opening of *Siren* is accurately located by H. H. WILDER (1889—1891) at the outer angle of the mouth between the antorbital cartilage and the pterygo-vomerine bar. FISCHER (1864, p. 114) describes the choana as a triangular opening provided with three valves, which he distinguished by the terms median, lateral and posterior. The posterior valve is said to be furnished with a small muscle by means of which the choana can be opened. FISCHER's median valve has also been observed by SEYDEL (1895, p. 507) and ANTON (1911, p. 191).

The median choanal valve of FISCHER corresponds to the choanal valve of *Necturus* and is easily identified both in living and preserved material. It is a narrow triangular fold of mucous membrane (Fig. 6), attached by one of its longer sides to the median margin of the opening, by its short side to the anterior margin. The so-called lateral valve of FISCHER does not exist.

The posterior choanal valve of *Siren* may be recognized in the living animal by its movements, which are caused by a small muscle

Fig. 7.

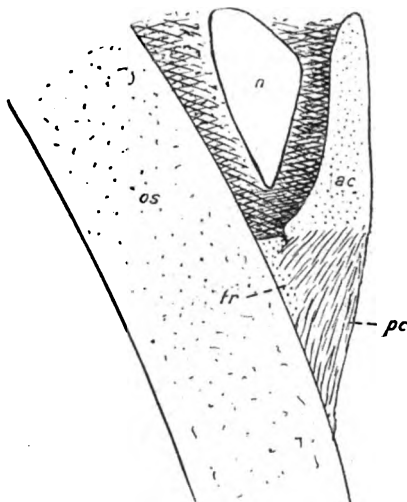


Siren lacertina. Transverse section through the posterior part of the choanal muscle, pc. On the median side of the muscle lies the cartilaginous trabecula cranii, from which many of the muscle fibres take their origin.

acting on the antorbital cartilage (Fig. 8). The latter is a horizontal plate of narrow triangular form, whose broad end is directed caudad, while its acute anterior end is embedded in the soft lip which forms the lateral margin of the choana (Fig. 6). The posterior median angle of this cartilage is attached by means of connective tissue to a remnant of the trabecula cranii which emerges from the orbito-sphenoid bone at the ventro-lateral angle of the brain case (Fig. 8). The antorbital cartilage is moved by a striated muscle, m. posterior choanae, which arises in part from the remnant of the trabecula cranii, in part from the adjacent surface of the orbito-sphenoid bone.

Its fibres run obliquely forward and laterad and insert on the postero-lateral angle of the antorbital cartilage. By the contraction of this muscle the anterior end of the cartilage is moved laterad and the

Fig. 8.



Siren lacertina. Choanal region from above (somewhat diagrammatic). *ac* antorbital cartilage; *n* choana; *os* orbito-sphenoid; *pc* m. posterior choanae; *tr* trabecula cranii.

choana becomes a large triangular opening which can not be covered by the median valve.

The choana is closed by the joint action of the median and posterior valves. When the m. posterior choanae relaxes, the elasticity of the adjacent tissues causes a return movement of the cartilage and valve, but the return is probably accelerated by the adductor muscles of the mandible which lie lateral and posterior from the choana. External pressure in this region in a recently killed specimen causes prompt closing of the opening, and since the adductor muscles contract to close the mouth during the act

of inspiration, they probably press on the posterior valve at this time. When the valve returns to its median position the choana is easily covered and protected by the median valve.

The character of the posterior choanal valve of *Siren* makes possible the expulsion of water from the mouth through the nasal cavity and explains the currents which occasionally stream from the nostrils during branchial respiration.

The Choanal Mechanism of *Amphiuma*.

A valve-like fold at the choanal opening of *Amphiuma* is mentioned in a casual way by CUVIER (1827, p. 5) and WAGLER (1830, p. 314) whose observations were accepted, apparently without further examination, by FISCHER (1864, p. 114). The description of the latter is as follows: »Vor jeder Öffnung liegt eine Falte der Gaumenhaut, die sich leicht nach hinten so über die Öffnung legt, daß letztere vollständig geschlossen wird.« A similar statement is made by COPE (1889, p. 216).

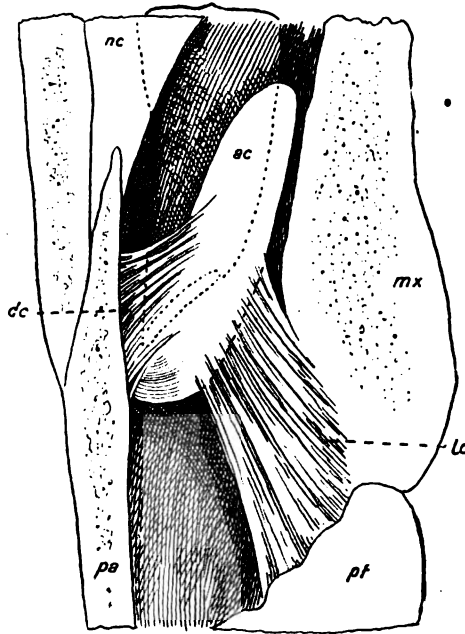
Hitherto undescribed choanal muscles in *Amphiuma*.

Since the expiratory currents pass freely through the nasal cavity of *Amphiuma*, it is evident that the choana is not closed in a purely mechanical way, as assumed by earlier authors. The opening lies at the posterior end of a short choanal canal, whose floor is formed by a membranous fold, the so-called valve of CUVIER. The choana is closed by depression of the roof of the choanal canal until it meets the ventral fold. By elevation of the roof of the canal the choana can be so widely opened that it can not be closed by the fold.

The mechanism which opens and closes the choana includes the antorbital cartilage and two striated muscles. The antorbital cartilage (Figs. 9, 10, 11) is a horizontal bar which arises by a short fibrous stalk from a remnant of the trabecula cranii which is exposed on the lateral surface of the orbito-sphenoid bone. From its attachment it extends forward and laterad on the dorsal aspect of the choana

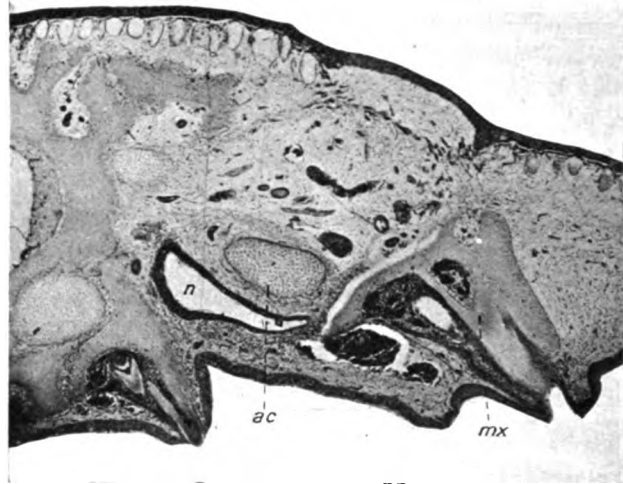
and the choanal canal. The distal end of the cartilage is elevated and depressed by the muscles above mentioned (Figs. 9 and 11). One of these, which I shall name *m. dorsalis choanae*, arises from the lateral wall of the cranium, chiefly from a slender process of the parietal bone which lies between the frontal and the orbito-sphenoid. From their origin the fibres of the muscle run ventro-laterad to insert on the dorsal surface of the antorbital cartilage. This muscle elevates the distal end of the cartilage and the roof of the choanal canal and thus opens the choana. The antagonist

Fig. 9.



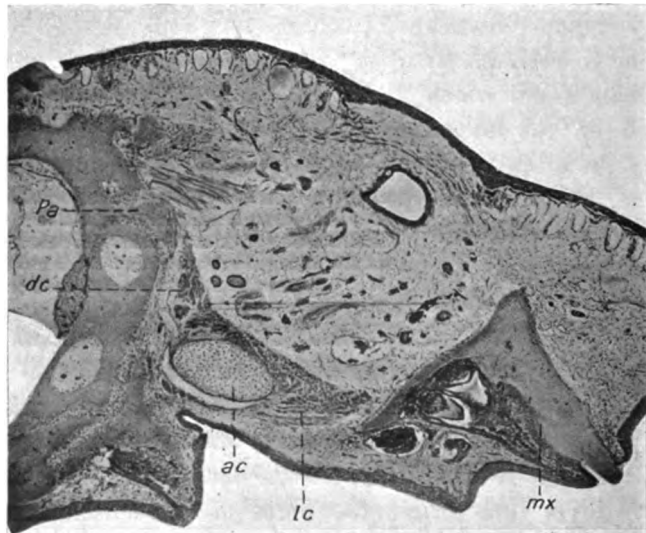
Amphiuma tridactylum. Choanal region, viewed from above. *ac* antorbital cartilage; *dc* *m. dorsalis choanae*; *lc* *m. lateralis choanae*; *mx* maxillare; *nc* choanal canal, which terminates in the choana beneath the antorbital cartilage and the *m. dorsalis choanae*; *nc* cartilaginous nasal capsule; *pa* parietale; *pt* pterygoid cartilage.

Fig. 10.



Amphiuma tridactylum. Transverse section through the anterior part of the choanal canal. *ac* antorbital cartilage; *n* choanal canal; *mx* maxillare.

Fig. 11.



Amphiuma tridactylum. Transverse section through the choana, *ac* antorbital cartilage; *dc* m. dorsalis choanae; *lc* m. lateralis choanae; *mx* maxillare; *pa* parietale.

of the *m. dorsalis choanae* is a somewhat stronger muscle, *m. lateralis choanae*, whose fibres arise in part from the palatine portion of the maxillary bone, in part from the anterior end of the pterygoid cartilage. The fibres run forward and mesad and insert on the postero-lateral surface of the antorbital cartilage.

Since *Amphiuma* is provided with two mechanisms for closing and opening the nasal passage, a question arises in regard to the function of each. On this point the following may be said: During aquatic respiration the muscles of the nasal vestibule are inactive and the introductory passage remains wide open. When the floor of the mouth is elevated the choana is usually closed. Occasionally it may be kept open to permit the escape of water through the nasal cavity. In pulmonary breathing the muscles of the nasal vestibule are active, the constrictor muscle contracting during inspiration, as it does in the higher urodeles. In expiration both choana and nasal vestibule are open and the air passes through the nasal cavity. Evidently the muscles of the nasal vestibule may also be used to protect the nasal cavity, for example, during the process of burrowing.

The Closing of the Choana in *Cryptobranchus*.

During pulmonary respiration the nasal passage is closed by the smooth muscle mechanism which lies at the inner end of the short nasal vestibule. In aquatic bucco-pharyngeal respiration the nasal vestibule remains wide open, but the loss of water through the nasal cavity is prevented by the closing of the choana. This is effected by the ceratohyal whose broad anterior portion is pressed against the oval opening when the floor of the mouth is elevated. An important part in the process is played by a cushion-shaped swelling which lies on the lateral side of the opening and was described by FISCHER (1864, p. 114) as a valve. It receives the lateral border of the ceratohyal when the floor of the mouth is elevated and thus aids in closing the choana. This method of closing the choana is possible in aquatic respiration because the oscillations of the floor of the mouth do not have great amplitude and the hyoid is never far removed from the choana. During pulmonary respiration the entire floor of the mouth is strongly depressed in aspiration and the choana remains open until a late stage of inspiration, if it is closed at all.

The Choanal Valve of Larval Amphibians.

The choanal opening of the *Amblystoma* larva has been studied in an incidental way by WAGLER (1830, p. 316) and CALORI (1851), the former describing two so-called choanal valves while the latter recognized but one. In my own preparations I find a single valve which resembles in all essential particulars the choanal valve of *Necturus*. In a larval *Amblystoma* 14 mm. long the valve is already recognizable as a slight fold on the median side of the almost circular opening. It evidently reaches a functional condition very early in larval life. In *Salamandra maculosa* it seems to be fully formed at birth, the larva having a length of about 25 mm. at this time. In *Triton alpestris* the valve seems to be functional in a larva of 18.5 mm. and it persists in all later stages examined, including a specimen 31 mm. long. In the large larvae of *Amblystoma tigrinum* the valve has been observed in specimens varying from 80 mm. to 240 mm. in length.

My material does not cover the last stages of larval life. It is certain, however, that the choanal valve is a temporary structure which disappears during the metamorphosis when the choana assumes the peculiar form which is characteristic of the adult. During the later larval stages, and before the choanal valve is lost, a new mechanism for closing and opening the nasal passage makes its appearance at the external naris. This is the smooth muscle mechanism of the adult salamander. The development of this mechanism, which includes a constrictor muscle and one or two dilators, has been described in a former paper (BRUNER, 1901, p. 328). Early stages of development occur in a larva of *Triton alpestris* 22 mm. long and the muscles are apparently functional in a specimen of 31 mm., which however still retains all of the larval characteristics. In later larval life, therefore, the salamander is provided with two mechanisms for closing the nasal passage.

In the preceding account I have mentioned only the larvae of lungbearing forms. In the lungless *Spelerpes ruber*, *Desmognathus fusca* and *Salamandrina perspicillata* a choanal valve of the type described above is wanting in the larva but the choana is probably closed in some as yet undiscovered way. In *Spelerpes ruber* regular aquatic respiration occurs, water entering the external naris and passing out through the gill clefts, as observed in *Necturus*.

The choana of the frog larva is closed by a so-called choanal fringe similar to that which SCHULZE (1892, p. 58) found in the larva of *Pelobates*. In the bullfrog larva this fringe is a prominent

fold which completely surrounds the opening. Its anterior half is much thickened and more or less rigid and thus forms a support for the posterior half which is thin and flexible and acts as a valve.

Summary and Comment.

1. The mechanism of pulmonary respiration in amphibians with gill clefts is a force-pump mechanism similar to that of higher amphibians.

2. Definite arrangements exist in all forms for the control of the respiratory media, including apparatus for closing the mouth, gill clefts and nasal passages.

3. The simplest and most primitive form of this respiratory mechanism is found in *Necturus*, *Proteus* and the larvae of higher lungbearing urodeles, a common characteristic of which is a mechanical choanal valve which prevents the escape of the respiratory media from the mouth through the nasal cavity. In all of these forms respiratory air is taken by the open mouth.

4. In *Siren* the respiratory mechanism is similar to that of *Necturus* but somewhat more complicated. Two choanal valves are present, a median valve corresponding to that of *Necturus*, and a posterior valve which encloses the antorbital cartilage and is moved by a small muscle. By means of the posterior valve the choana can be opened and expiration through the nasal cavity is possible.

5. *Amphiuma* is provided with smooth muscles for opening and closing the nasal vestibule and with a complicated choanal mechanism, which includes the antorbital cartilage and two striated muscles. By means of these muscles the choana can be opened and closed. Respiratory air is taken through the nasal cavity, as in higher urodeles.

6. In *Cryptobranchus* the choana is closed by the hyoid arch. Respiration, both bucco-pharyngeal and pulmonary, is similar to that of *Amphiuma*.

7. The character of the respiratory mechanism of the *Perennibranchiata* seems to indicate a) that *Necturus* and *Proteus* are slightly modified permanent larvae, b) that *Siren* is a modified larva whose choanal mechanism has been brought under muscular control.

8. The *Cryptobranchiata* appear to be much modified permanent larvae whose development has been arrested during the metamorphosis. They have retained the essential parts of the aquatic respiratory mechanism excepting the gills, while at the same time they have the pulmonary mechanism of the higher urodeles.

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AN ENTOMOLOGIST IN COSTA RICA.*

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Until the separation of Panama as an independent state from Colombia, Costa Rica was the southernmost of the five republics of Central America. It lies between Nicaragua on the north and Panama on the south, from latitude 11° to 8° North. Its general trend is from northwest to southeast, and through its entire length runs a series of peaks, many of them volcanoes, whose greatest altitude is above 12,000 feet. North of the 10th parallel, this chain divides into two branches one of which, extending in a more easterly direction toward the Atlantic, is composed chiefly of the volcanoes Poas (8786 ft.), Barba (9508 ft.), Irazu (11326 ft.) and Turrialba (10965 ft.). The other branch, retaining the southeastward trend, is continued by the Cordillera of Chiriqui in Panama and includes the highest elevations in the country. Along the 10th parallel the distance from the Atlantic to the Pacific is 185 miles, but if we measure to the eastern shore of the Gulf of Nicoya, that is from the port of Limon to Puntarenas, 125 miles. The railroad in making this transit climbs to 5000 feet and this ascent together with its windings increases the actual distance to 175 miles.

The prevailing easterly trade winds coming from the Caribbean, laden with moisture, strike against the lofty mountains and cause a heavy precipitation on the Atlantic slope throughout much of the year. Sheltered by the same peaks the Pacific slopes and even some localities on the Atlantic, like Cartago, receive a smaller precipitation until southerly winds bring moisture from April to November.

*Abstract of address before the Entomological Society of America, Cleveland, Ohio, Jan. 1, 1913. The address was illustrated by a very fine series of lantern views from photographs of insects and localities of scientific and scenic interest.
—Ed.

Passing from east to west, the average annual rainfall at Limon is 126.8 inches, Juan Viñas 85.6, Cartago 60.7, with minimal average monthly precipitations of 5, 2.5 and 1 inch respectively (all these on the Atlantic slope), while corresponding figures on the Pacific slope are 76 inches for Tres Rios, 76.4 for San Jose and 62.1 for Nuestro Amo, the minimal average monthly rainfalls being .12, .43 and 0 inches respectively.

The abundant rainfall gives rise to many streams of all sizes. Erosion and the undermining of the loose soil have cut the surface of the land into many deep ravines and canyons, producing a rugged topography and making travel difficult and time-consuming. Within short horizontal distances are great differences of elevation. This, in turn, has affected the character of the vegetation and of the fauna. Pronounced segregation of many living things is consequently often the case, and the richness of the biota, as estimated by the number of species, is greatly increased.

Pittier, in 1908, gave the number of species of flowering plants of Costa Rica as 3441; the corresponding number for New Jersey is 1351 (Stone, 1910). Carriker, in 1910, listed 753 species and subspecies of birds from Costa Rica, or more than half the total number (1196) for America north of Mexico in the A. O. U. check list of the same year, and twice as many as have been recorded in recent years for Maine (327), Colorado (392) or Washington (372); the smallest of these three has an area at least a third greater than that of Costa Rica which is only 23,000 square miles. Rehn, in 1905, gave a partial list of 195 species of Costa Rican Orthoptera, as against 154 species in the far more thoroughly explored state of New Jersey. Godman and Salvin, in 1901, enumerated 236 genera of Costa Rican butterflies; Dyar, in 1902, recognized 152 genera for America north of Mexico. Schaus has found 150 Costa Rican species of the butterfly genus *Thecla*, as contrasted with 56 species in America north of Mexico.

All of these characteristics make Costa Rica a Paradise to the naturalist. Its variety of altitude offers variety of temperature. The short distance from the shores of the Atlantic to those of the Gulf of Nicoya, an arm of the Pacific, and the existence of the transcontinental railroad render it possible to pass from one to the other in ten hours; a comparison of conditions at similar altitudes on the two slopes of the divide may be easily

and quickly made. In the higher parts of the country the climate is salubrious and invigorating, and with a little care one may safely investigate the heated lowlands. Proximity to South America, with no intervening barrier, has permitted the invasion of many denizens of the Southern Continent, while not a few cases of continuous distribution from North America are also in evidence. The most orderly of Central American countries holds its presidential elections with as much enthusiasm and with less disturbance than those of the United States. A peaceful and hospitable people and an enlightened government render the stranger's visit an event to be remembered by him with delight throughout a lifetime.

In one or other of these qualities, Costa Rica is excelled by Mexico, Colombia or Brazil, but by none in the totality of the advantages which it offers to the students of all the branches of ecology in its widest sense. One shadow, indeed, hangs over the fair land—that of the earthquakes which within two centuries have thrice destroyed the town of Cartago, lying on the southern slopes of the volcano Irazu, the latest destruction being that of May 4, 1910, when it was serving as our own headquarters.

During the year, May 1909, to May, 1910, insects, especially Odonata (dragonflies) were collected and studied at the following fourteen groups of places and at intervals, in order to obtain data on seasonal distribution.

On the Atlantic slope:

Banana River region, 50 feet, November.

Guapiles, 984 feet, June, November.

Peralta, 1088 feet, August, March.

Turrialba, 2000 feet, July.

Juan Viñas, 2500–4000 feet, June, August, October,
December, February, March April.

Cachi, 3600 feet, March.

Cartago, 4750 feet, every month.

Volcano Irazu, 4750–11300 feet, July, September, March.

On the Pacific slope:

Tres Rios and La Carpintera, 4260 to 5700 feet, December, March.

Alajuela, 3100 feet, September, December.

Turrucares, 1800–2200 feet, August, December, April.

Surubres, 800 feet, October.

Puntarenas, 10 feet, February.

Guanacaste, 0–2200 feet, January.

Four of these localities are here described briefly.

Juan Viñas, on the Atlantic slope, was particularly fruitful as a collecting ground owing to its combination of many of the advantages mentioned above. The railroad station, 73 miles from Limon, and at an altitude of 3300 feet, is on the bottom of an old crater the rim of which, at the general level of the country, is 700 feet higher; the village of Juan Viñas is at this latter elevation. From the railway, in half an hour, one may reach the Rio Reventazon, 800 feet below. The canyon of this river thus has a depth of 1500 feet, and presents a great variety of slow- and of swift-flowing brooks, cascades, waterfalls, forest, swamp, bare rock and dense vegetation. It was productive of material illustrating previously unknown life-histories of interesting Odonata (*Cora*, *Mecistogaster*, *Thaumatoneura*, *Philogenia*, *Palaemnema*, etc.).

Surubres, on the Pacific side, at an altitude of about 800 feet, was a favorite with the late Professor Paul Biolley, where he gathered much insect material subsequently sent to entomologists in the United States and in Europe. A week was spent in the hacienda, which he occupied on several occasions, but at a different time of year, to secure data to supplement those which he obtained.

The northwestern province of Costa Rica, Guanacaste, has been little visited by entomologists. Thanks to Professor J. F. Tristan, the writer accompanied an official educational commission thither, and collections and observations were made at Filadelfia, Liberia, Santa Cruz, and Hacienda Guachipelin. The last named, at an altitude of 1700 feet, is not far from the still unexplored Volcano Rincon de la Vieja.

Cartago, near the top of the Atlantic slope of the railroad, was, until its destruction, alluded to above, a convenient center for visits to various parts of the country and served as the breeding place of living material collected on these excursions.

(Other aspects of this visit to Costa Rica have been described in *Entomological News*, vol. XXI, pp. 334-337, July, 1910, and in *Old Penn Weekly Review of the University of Pennsylvania*, vol. IX, pp. 165-170, Nov. 12, 1910. Some of the results obtained from studies on Costa Rican Odonata have been published in *Entomological News* for 1910, 1911 and 1912, and will probably be continued in subsequent volumes of the same journal.)

**THE FOSSIL ODONATE PHENACOLESTES, WITH A DISCUSSION OF THE
VENATION OF THE LEGION PODAGRION SELYS.**

BY PHILIP P. CALVERT.

CONTENTS.

Phenacolestes Material Studied, p. 225.
The Principal Characters of Phenacolestes, p. 227.
Comparisons of the fossils Dysagrion (p. 230), Melanagrion (p. 231) and Lithagrion (p. 232) with Phenacolestes.
Comparison of Thaumatozona with Phenacolestes, p. 233.
Relationships of Thaumatozona, p. 233.
Comparisons of Philogenia (p. 235), Megapodagrion [Allopodagrion] (p. 236), Paraphlebia (p. 237), Dimeragrion (p. 238), Heteragrion (p. 239), Mesagrion, Heteropodagrion and Neuragrion (p. 240), Rhipidolestes (p. 241), Podopteryx (p. 242), Argiolestes (p. 243), Chlorolestes (p. 244) and Various Old World Genera (p. 244) with Phenacolestes.
General Discussion of the Value of the Venational Characters compared in the preceding Genera for the Determination of Relationships, p. 245.
Synopsis of Venational Characters of Genera of the Legion Podagrion, p. 256.
Outline of an Alternative Grouping of the Genera of the Legion Podagrion, p. 261.
The Relationships of Phenacolestes, p. 262.
On the Subfamily Dysagrioninae Cockerell, p. 264.
Additional Data on Dimeragrion, n. gen., p. 265.
Bibliography, p. 270.
Explanation of Plate XIV, p. 272.

PHENACOLESTES MATERIAL STUDIED.

In 1908, Professor T. D. A. Cockerell described (1908a) two species of fossil Odonata from Station 14, Florissant, Colo., as *Phenacolestes mirandus* and *Ph. (?) parallelus*. A little later (1908b) he added a note based on an additional fragment which he referred to the latter species, and in another place (1908c) he gave a figure showing the body as well as the wings, the previous accounts having referred to the wings only. In connection with the original description he quotes from a letter from Professor Needham who almost implies that *Ph. mirandus* is to be placed in de Selys' legion Podagrion of the Agrioninae (1908a, p. 63).

In the latter part of 1910, Professor Cockerell wrote to me that he had found more wings of *Phenacolestes*, and requested me to study them, in view of some differences of opinion as to its relationship to modern dragonflies.

When I accepted this offer, Professor Cockerell sent me four

specimens of these fossil wings which, for reference here, I have numbered as follows:

No. 1. Labelled "*Phenacolestes mirandus* Ckll. Florissant," in Professor Cockerell's handwriting; 27 mm. long from base to apex.

No. 2. Labelled "*Phenacolestes mirandus* Ckll. Florissant Sta. 12 B (G. N. Rohwer)," in the same hand. Apparently a hind wing. Distorted, length from base to apex as it lies on the stone 26 mm.

Nos. 3 and 4. Unlabelled. [These are the two impressions of the same wing, and were collected by Geo. N. Rohwer. T. D. A. Cockerell.] Apparently a front wing. Length of No. 3 from base to apex 28 mm.; of No. 4 (apex lost) from base to distal end of stigma 25 mm.

They are figured from enlarged photographs on Plate XIV as figures 1-4.

The beautiful preservation of these wings has rendered possible the drawing up of almost as full a description of the venation as for any living species.

Professor Cockerell has very kindly presented specimen No. 3 to the Academy of Natural Sciences of Philadelphia; the other three specimens will be returned to him.

Thanks to the kindness of Dr. F. A. Bather, through the good offices of Mr. C. J. Gahan, I have also studied two fossils of this insect in the British Museum of Natural History, when in London in August, 1912. One of these, catalogued as "No. I 8423," is the paratype of Professor Cockerell's original description of *Phenacolestes mirandus*. The other, "No. I 15,049," is labelled in Professor Cockerell's handwriting "*Phenacolestes mirandus* Ckll. Florissant Sta. 13 B (S. A. Rohwer)." No. I 8423 measures 20.5 mm. in length from the wing-base to the proximal end of the stigma; the distal part of the stigma and the tip of the wing are lacking; the nodus is 9 mm. from the base. No. I 15,049 has lost the stigma and apex of the wing from the distal end of M_2 forward (cephalad and distad) and the extreme hind margin between the apices of M_2 and Cu_1 ; its length along the costa as preserved is 23 mm., the nodus is 10 mm. from base.

Use has also been made of an excellent photograph, for which I thank Professor Cockerell and Dr. Max M. Ellis, of the University of Colorado, of an almost perfect wing from the same locality as that of the second specimen quoted in the preceding paragraph. This photograph is reproduced as figure 5 on Plate XIV accompanying this paper; it will be designated as No. 5 in these pages and apparently represents a front wing.

The following account is based, therefore, on six wings in seven impressions.

Throughout this paper the Comstock-Needham nomenclature for the wing-veins has been employed. In the previous literature on the living members of the legion Podagrion the Selysian terms have been used. The equivalents of the two systems are therefore appended here.

De Selys: Costa	=	C.-N.: Costa, C.
Subcosta	=	Subcosta, Sc.
Median vein	=	R ₁ .
Principal sector	=	M ₁ .
Ultra-nodal sector	=	M _{1a} .
Nodal sector	=	M ₂ .
Subnodal sector	=	R. (+ the bridge, if it be present). ¹
Median sector	=	M ₃ .
Short sector	=	M ₄ .
First (or superior) sector of the triangle	=	Cu ₁ .
Second (or inferior) sector of the triangle	=	Cu ₂ and Anal (A).
Basal postcostal nervule	=	Cubito-anal cross-vein or c-a.

THE PRINCIPAL CHARACTERS OF PHENACOLESTES (Plate XIV, figs. 1-5).

1. Nodus at one-third of the wing-length.
2. More than two antenodals. (Four in at least two (Nos. 2, 3, 4) of these specimens, five in No. 5 and in the two cited from Brit. Mus.)
3. Postnodals 17-19.
4. Stigma having its proximal edge oblique.
5. Cells of the wing generally, posterior to M₁, not greatly elongated at right angles to long axis of wing.
6. The veins generally, posterior to M₁, have but a slight caudal curvature shortly before they approach the hind margin of the wing.
7. Between M₁ and M_{1a} is only one row of cells throughout to wing-margin. (In No. 5, increased to two rows at five cells' distance from the margin.)
8. Between M_{1a} and M₂ is one row of cells, increasing to two and then three rows, and so continuing to wing-margin.

¹ Throughout this paper "Rs" has been used as the equivalent of de Selys' "subnodal sector."

9. M_2 separates from M_1 at five-thirteenths ($= .38$) of the distance from nodus to stigma. (In No. 5, however, the distance is only .28, in No. I 8423 B. M. it is .26.)
10. Between M_2 and R_1 is one row of cells increasing to two and then three rows, and so continuing to the margin.
11. R_1 separates from $M_1 + 2$ at the subnodus. (Nos. 3, 4, 5, and B. M. I 15,049 show this clearly; B. M. I 8423 apparently agrees with them; No. 1 has the point of separation slightly proximal to the subnodus, while in No. 2 this part is indistinguishable.)
12. Between R_1 and M_2 is one row of cells, increasing to two and then to three rows of cells.
13. Arculus slightly proximal to the second antenodal.²
14. M_2 separating from $M_1 + 2$ at six-tenths' to three-fourths' way from arculus to subnodus.
15. Between M_2 and M_4 are (a) at least two cross-veins proximal to the level of the subnodus,³ and (b) at the wing-margin are 4-5 rows of cells.
16. Quadrilateral with (a) its proximal and distal sides strongly diverging caudad and (b) its distal side more than one-and one-half times as long as its proximal side.
17. Between M_4 and Cu_1 are (a) seven antenodal cells (*sensu Selysi*) and (b) at the wing-margin four to five rows of cells.
18. Cubito-anal cross-veins one, which is situated between the levels of the first and second antenodals.
19. Between Cu_1 and Cu_2 at the wing-margin are about two rows of cells (three rows in B. M. No. I 15,049).
20. Area posterior to Cu_2 without distinct branches, but with three to four rows of cells.
21. Anal vein separating from the hind wing-margin (a) proximad to the level of the arculus and (b) proximad to the level of the cubito-anal cross-vein.
22. Anal cross-veins, proximal to the level of the distal end of the quadrilateral, one.

Compared with Professor Cockerell's original description of *Phenacolestes mirandus* (1908a, p. 61), the present material shows the following differences:

Antenodal cross-veins⁴ four in *some* specimens instead of five.

Basal space (*i.e.*, cell $M_1 - 4$) 4 mm. long, instead of nearly 5 mm.

² For B. M. No. I 8423 I have even noted, "Arculus very slightly more distal than midway between the two normal antenodals."

³ The second, or distal, of these is actually at the subnodus in the two British Museum specimens.

⁴ Prof. Cockerell speaks of antenodal and postnodal *sectors* meaning these cross-veins. This word is not in accordance with the usage of other authors on the Odonata. R_3 in the same description should be R_2 .

Quadrangle with its upper side distinctly shorter than the lower side.

M₂ separating from M₁₊₂ at 3.5–4 mm. from the arculus, 2.5–1 mm. from level of nodus.

Postnodal cross-veins⁴ 17–19, instead of 21.

Stigma bounding $3\frac{1}{2}$ or $4\frac{1}{2}$ cells above, preservation imperfect in most of these specimens.

M₁ and M₂ separating at as much as 10.75 mm. from arculus, instead of 9.67 mm.

Beyond the quadrangle, *i.e.*, between M₄ and Cu₁, are seventeen single cells (Nos. 3 and 4), or sixteen (No. 5), then two rows increasing; hence very different from Prof. Cockerell's statement, p. 62, which does not seem to agree with his figure (13 of Plate V).

There is but one row of cells between M₁ and M₁₂ throughout, thus agreeing with the description of *Ph. (?) parallelus* (*l.c.*, p. 62) in distinction from *Ph. mirandus*.

Two specimens, Nos. 1 and 3, are sufficiently preserved to enable a count of the cells between M₂ and R₄ to be made. From the margin proximad these are, in No. 1, 7 marginal cells, then 3 rows for 3–4 cells, then 2 rows for 6 cells, then 1 row (which is consequently met at 10–11 cells in from the margin), this single row comprising about 15 cells to the origin of R₄, three of these 15 being basad of the origin of M₂. Corresponding figures for specimen No. 3 are 5 (?) marginal cells, then 3 rows for 4 cells, then 2 rows for 3 cells, then 1 row (which is consequently met at 8 cells in from the margin), this single row comprising about 20 cells to the origin of R₄, $5\frac{1}{2}$ of these 20 being basad of the origin of M₂. In B. M. No. I 8423, this single row consists apparently of 15 cells, 4 of which are basad of the origin of M₂. In B. M. No. I 15,049, this single row consists of 15 or 16 cells, 3 and the greater part of the fourth of which are basad of the origin of M₂; No. 5 appears to be very similar. None of these arrangements is identical with that described for *Ph. mirandus*, where, however, the single row of cells is met at 11 cells in from the margin.

Prof. Cockerell gives two formulæ for *Ph. mirandus* and *Ph. (?) parallelus*, respectively (*l.c.*, p. 62), these formulæ being obtained by taking "a section of the wing down from the basal part of the stigma to Cu₂, and count[ing] the numbers of rows of cells successively met between the longitudinal veins . . . commencing with that between R₁ and M₁." The numbers of cells so obtained will depend on the exact position of the line of section, but Prof. Cockerell does not specify this. Choosing, as the two points fixing this line, the

inferior proximal angle of the stigma and the point where Cu₂ meets the hind wing-margin, the following formulæ for the present material were obtained:

No. 1: 1, 1, 2, 1, 2, 2, 3, 2.

No. 2: (too imperfect).

No. 3: 1, 1, 2, 1, 2, 2, 3, 2.

No. 4: 1, 1, 2, 1, 2, 2, 3, 2.

No. 5: 1, 1, 2, 2, 2, 2, 3, 2.

No. I 15,049: 1, 1, 2, 2, 2, 2, 3, 2.

We have here two different formulæ, neither of which agree with either of Prof. Cockerell's formulæ.

Guided by the amount of variation in venational details of one and the same living species of Agrioninæ, I am of the opinion that the differences shown by the present material, *inter se* and from Prof. Cockerell's data, do not justify the specific separation of the specimens before me from *Ph. mirandus*.

All of the present material show more or less distinctly the dark band across the wing, described by Prof. Cockerell; it is best illustrated in our figure 5.

Phenacolestes agrees with the venational characters of the legion Podagrion, as stated by de Selys in 1886,⁵ with the one exception that the wings are *not* "pétiolées beaucoup plus loin que la nervule basale postcostale." I think that there can be no doubt that its nearest living allies are to be found in this group. I have therefore made comparisons with a number of genera of this legion, especially American, with the results set forth below. As I have had only five of the fossil wings and photographs of one other before me and am unable to determine whether all of them are of the fore or hind pairs, I have not thought it necessary to employ any great number of specimens of the living species for these comparisons.

COMPARISON OF THE FOSSIL DYSAGRION (PACKARDII, FREDERICII) WITH PHENACOLESTES.

According to the figures and descriptions of this Eocene genus, from the Green River shales of Wyoming, given by Scudder,⁶ *Dysagrion* and *Phenacolestes* agree in the characters above numbered 2, 3, 4, 5, 7,⁷ 10, 11,⁸ 14, 15b, 16a and b, 20, 21a and b, and 22, and

⁵ *Mem. Couron. Acad. Belg.*, XXXVIII, p. 30.

⁶ *Tertiary Insects of North America*, 1890, pl. 6, figs. 3, 9, 14 and pp. 128-133.

⁷ In *D. packardii*, but not in *D. fredericii*.

⁸ As Scudder has pointed out, p. 129, however, the subnodus is bent proximad (instead of distad as is the case in *Phenacolestes*).

differ in the following characters, the difference shown by *Dysagrion* being stated:

1. Nodus at .37 of the wing-length.
6. The veins generally, posterior to M_1 , have a slighter caudal curvature as they approach the hind margin.
7. Between M_1 and M_{1a} are two rows of cells beginning under the stigma (*D. fredericii*, not *D. packardii*).
8. Between M_{1a} and M_2 is one row of cells increasing to two, three, and finally five rows.
9. M_2 separates from M_1 at .15° of the distance from nodus to stigma.
12. Between R_1 and M_3 is one row of cells increasing to two, three, and eventually eight rows.
13. Arculus apparently at the second antenodal.
- 15a. Between M_3 and M_4 is apparently only one cross-vein proximal to the level of the subnodus.
17. Between M_4 and Cu_1 are (a) 8–10 antenodal cells (the quadri-lateral is much shorter than in *Phenacolestes*) and (b) at the wing-margin only one row of cells.
18. Cubito-anal cross-veins two in *D. fredericii*, one of them proximal, the other distal, to the arculus; none shown in *D. packardii*.
19. Between Cu_1 and Cu_2 at wing-margin one (*D. packardii*) or three (*D. fredericii*) rows of cells.

COMPARISON OF THE FOSSIL MELANAGRION (UMBRATUM) WITH PHENACOLESTES.

According to the figures and description of this Miocene genus from Florissant, Colo., given by Scudder,¹⁰ *Melanagrion* and *Phenacolestes* agree in the characters above numbered 5, 7, 8,¹¹ 9, 10,¹¹ 13, 14, 15a, 16a and b, 18, and differ in the following characters, the difference shown by *Melanagrion* being stated:

1. Nodus at .3 (?) of the wing-length.
2. Only two antenodals.
3. More than 26 postnodals (27, Scudder).
4. Stigma having both proximal and distal ends much less oblique.
6. The veins generally, posterior to M_1 , have a slighter caudal curvature as they approach the hind margin.
11. R_1 separates from M_{1+2} slightly proximal to the subnodus.

⁹ This is taken by measurement from Scudder's figure, although he says that the "nodal sector arises from the principal . . . scarcely more than one-fifth way to the pterostigma" (p. 129), and in another place (p. 128) "at scarcely one-fifth the distance from the nodus to the pterostigma." Whether figure or text be correct, both differ from the condition in *Phenacolestes*.

¹⁰ Scudder, *Tertiary Insects of North America*, 1890, pl. 13, figs. 12, 14, p. 136, described this form as a *Lithagrion*. It was made the type of a new genus, *Melanagrion*, by Prof. Cockerell in *Bull. Amer. Mus. Nat. Hist.*, XXXIII, p. 138, 1907.

¹¹ At the extreme margin there are four rows in *Melanagrion*.

12. Between R_1 and M_3 is one row of cells increasing to two rows.
- 15b. Between M_3 and M_4 at the wing-margin are only three rows of cells.
17. Between M_4 and Cu_1 are (a) about three antenodal cells and (b) at the margin one row of cells.
19. Between Cu_1 and Cu_2 at the wing-margin appear to be but one row of cells.
20. Area posterior to Cu_2 with no more than two rows of cells.
21. Anal vein separating from the hind wing-margin (a) distad (fig. 12) or proximad (fig. 14) to the level of the arculus and (b) at the level of the cubito-anal cross-vein (fig. 14, cross-vein not shown in fig. 12).¹²
22. No anal cross-veins, according to both figures.

COMPARISON OF THE FOSSIL LITHAGRION (HYALINUM) WITH
PHENACOLESTES.

According to the figure and description of this Miocene genus from Florissant, Colo., given by Scudder¹³ and some notes on more recently found specimens kindly communicated by Prof. Cockerell, *Lithagrion* and *Phenacolestes* agree in the characters above numbered 1, 3, 5, 7, 8, 9, 10 (probably), 11, 14, 15a, 16a and b, 18, and differ in the following characters, the differences shown by *Lithagrion* being stated:

2. Probably only two antenodals.¹⁴
4. Stigma having its proximal edge very much less oblique.
6. The veins generally, posterior to M_1 , have a slighter caudal curvature as they approach the wing-margin.
12. Between R_1 and M_3 is only one row of cells to the wing-margin.
13. Arculus at the second antenodal.¹⁵
- 15b. Between M_3 and M_4 at the wing-margin are only two or three rows of cells.
17. Between M_4 and Cu_1 are (a) three antenodal cells and (b) one row of cells increasing to two rows only at the extreme margin.
19. Between Cu_1 and Cu_2 at the wing-margin is only one row of cells.
20. Area posterior to Cu_2 with only one row of cells.
21. Anal vein separating from the hind margin of the wing distad to the levels (a) of the arculus and (b) of the cubito-anal cross-vein.
22. Anal cross-veins proximal to the level of the distal end of the quadrilateral apparently none.

¹² Scudder's description, p. 136, says, "The wings are petiolated very nearly up to the first postcostal nervule, which is placed shortly before the base of the quadrilateral."

¹³ *Tertiary Insects of North America*, 1890, pl. 13, fig. 4, and pp. 128, 134-136.

¹⁴ Scudder's figure, i.e., shows but one, at the arculus. Compare Prof. Cockerell's statements on this point in *Bull. Amer. Mus. Nat. Hist.*, XXXIII, p. 137 (1907); XXXIV, p. 63 (1908); *Amer. Journ. Sci.*, XXVI, p. 69 (1908).

¹⁵ A sketch kindly sent by Prof. Cockerell shows the arculus a little distal to the second antenodal.

COMPARISON OF THAUMATONEURA (INOPINATA AND PELLUCIDA)
WITH PHENACOLESTES.

(*Th. inopinata* ♂ ♀, *Th. pellucida* ♂, Costa Rica).

These two genera agree in the characters above numbered 2, 11, 14 (except as noted below), and 21a. As regards character 11, the separation point of R_1 in *Thaumtoneura* varies and may be a little proximal to, at, or a little distal to, the subnodus.

The two genera differ in characters given below; after each number the difference shown by *Thaumtoneura* is stated:

1. Nodus at about one-fifth of the wing-length.
3. Postnodals very numerous, about 70.
4. Proximal edge of stigma more oblique.
5. Cells more elongated at right angles to long axis of wing.
6. Stronger caudal curvatures of the veins generally as they approach the hind margin of wing.
7. The one row of cells between M_1 and M_{1+2} increases to about 10 rows at the wing-margin.
8. The one row of cells between M_{1+2} and M_2 increases to 6+ rows at the wing-margin.
9. M_2 separates from M_1 at nearly one-half way from nodus to stigma and at a less acute angle.
10. Between M_2 and R_1 at the wing-margin are 6-7+ rows of cells.
12. Between R_1 and M_3 at the wing-margin are 7-8+ rows of cells.
13. Arculus at the second antenodal.
14. M_3 separates from M_{1+2} at a less acute angle.
15. Between M_3 and M_4 , (a) no cross-veins proximal to the level of the subnodus, (b) at the wing-margin 10+ rows of cells.
16. Quadrilateral with (a) its proximal and distal sides subparallel and (b) subequal.
17. Between M_4 and Cu_1 are (a) 3-6 antenodal cells and (b) at the wing-margin 8+ rows of cells.
18. Cubito-anal cross-veins two, one proximal to the level of the first antenodal cross-vein, the other below the middle of the quadrilateral.
19. Between Cu_1 and Cu_2 at wing-margin are about 12 rows of cells.
20. Area posterior to Cu_2 much larger, Cu_2 with many branches.
- 21b. Anal vein separates from the hind margin of the wing distad to the level of the proximal cubito-anal cross-vein.
22. Anal cross-veins proximal to the level of the distal end of the quadrilateral 3-5.

RELATIONSHIPS OF THAUMATONEURA.

Some words may be added here as to the retention of *Thaumtoneura* in this group after the diverging opinions of Professors Needham and Foerster. The late Mr. McLachlan, on describing *Thaumato-*

neura (1897), referred it to the Calopteryginæ. In 1902, I placed it in the legion Podagrion of the Agrioninæ.¹⁶ In 1903, Professor Needham associated it with the Anormostigmatini (= legion Pseudostigma of Selys), stating that "it has all the essential venational characters of this group, and surely these are sufficiently distinctive."¹⁷ A comparison of *Thaumatoneura* with these essential venational characters¹⁸ gives the following result.

Thaumatoneura agrees with the Anormostigmatini in that R₁ separates from M₁₊₂ at or very close to the subnodus and the part of the wing beyond the nodus is greatly enlarged. On the other hand, *Thaumatoneura* differs from the Anormostigmatini in that, while the stigma is not braced, it is not diffuse or lost; the matching of cross-veins is not so marked; the longest vein between M₁ and M₂, which, in the preceding pages, we have assumed to be M_{1a}, does not parallel M₂ more than it parallels M₁ and lies almost midway between M₁ and M₂; it has shorter supplementary sectors between itself and M₁ and also between itself and M₂; these latter tend to be longer and better developed than those anterior to M_{1a}, and in this *Thaumatoneura* resembles *Paraphlebia* and differs from the Anormostigmatini. This tendency to the development of more rows of cells between M_{1a} and M₂ than between M₁ and M_{1a} is emphasized in *Philogenia carrillica*, *P. terraba*, *Heteragrion tricellulare*, *H. erythrogastrum* and *Argiolestes icteromelas* which have no supplementary sectors between M₁ and M_{1a}, but two between M_{1a} and M₂; all these are Podagrionines. Even in more reduced venations of the same group, as in *Allopodagrion* there are no supplementary sectors between M₁ and M_{1a}, but still one between M_{1a} and M₂. On the other hand, even in Anormostigmatini with the most reduced venation, e.g., *Mecistogaster*, there is a longer supplementary sector between M₁ and M_{1a} than between M_{1a} and M₂. Professor Needham himself has pointed out some further differences of *Thaumatoneura* from *Megaloprepus*, and still others may be added. Thus the nodus and quadrilateral (quadrangle) are no more approximated in *Thaumatoneura* than in many other undoubted members of the legion Podagrion (See Table I, p. 250, *postea*). *Thaumatoneura* possesses more supplementary sectors between M₂ and R₁ and between R₁ and M₃. I believe, therefore, that *Thaumatoneura* shows more resemblances to the legion Podagrion than to the legion Pseudostigma, but it does not

¹⁶ 1902, pp. 29-32. *Biol. Centr. Amer. Neur.*, p. 58.

¹⁷ *Genealogic Study*, pp. 748-749, footnote.

¹⁸ Needham, l.c., p. 748.

seem impossible that the Anormostigmatini may have descended from some form like *Thaumatoneura*.

Professor Foerster considers *Thaumatoneura* as a connecting form between the Calopteryginæ and the legion Podagrion, "which is better left with the Calopteryginæ." "Bei *Thaumatoneura* ist der äussere Ast des Sector inferior trianguli convex nach vorn gebogen wie bei allen echten Calopterygiden. Bei allen Agrioninen ist er dagegen schwach convex nach hinten."¹⁹ Many Calopteryginæ (Selys) have not Cu_2 convex anteriorly, of course, such as *Micromerus*, *Rhinocypha biforata* and other species, *Libellago*, etc., but perhaps these are not "echt." On the other hand, such Agrionines as *Megaloprepus*, *Microstigma*, and *Paraphlebia* also have Cu_2 convex anteriorly, while a tendency in the same direction is observable in *Argiolestes* and even in *Hyponeura*, so that I cannot see that any strong argument for the Calopterygine affinities of *Thaumatoneura* can be drawn from this character.

COMPARISON OF PHILOGENIA (TERRABA, CARRILICA) WITH PHENACOLESTES.

(*Ph. terraba* ♂, *Ph. carrillica* ♂ ♀, Costa Rica.)

These two genera agree in the characters above numbered 4, 5, 6, 7, 10,²⁰ 11 (*carrillica*, not *terraba*), 12,²⁰ 14, 16, 18.

These two genera differ in characters given below; after each number the difference shown by *Philogenia* is stated.

1. Nodus at one-fourth of the wing-length.
2. Only two antenodals.
3. Postnodals 22-30.
8. Between M_{1a} and M_2 is at first but one row of cells which, more distad, is increased to two and then to three rows by the presence of two distinct curvilinear (i.e., not broken) supplementary sectors, the posterior of which arises proximad to the anterior; each of these three rows increases to a double row before reaching the wing-margin at an individually variable distance therefrom.
9. M_2 separating from M_1 at one-third of the distance from nodus to stigma.
11. R. separating from M_{1+2} a little distad to the subnodus (*terraba*, not *carrillica*).
13. Arculus slightly distal to the second antenodal.

¹⁹ *Jahrb. Nassau. Ver. f. Naturk. in Wiesbaden*, 62, p. 214, 1909.

²⁰ At the very margin itself, each of the three rows in *Philogenia* tends to divide into two rows, but each of these last extends inward only 2-3 cells from the margin.

15. Between M_3 and M_4 is (a) one cross-vein proximal to the level of the subnodus and (b) at the wing-margin are two or three rows of cells.
17. Between M_4 and Cu_1 are (a) 2-3 antenodal cells and (b) at the wing-margin one row of cells.
19. Between Cu_1 and Cu_2 at the wing-margin is one row of cells.
20. Area posterior to Cu_2 less developed, having only one row of cells.
21. Anal vein separating from the hind wing-margin at the mid-level of the quadrilateral, hence distad both to (a) the level of the arculus and (b) that of the cubito-anal cross-vein.
22. No anal cross-vein proximal to the level of the distal end of the quadrilateral, but there is a cross-vein continuing the vein which forms the distal end of the quadrilateral to the wing-margin; this cross-vein is absent in *Phenacolestes*, *Thaumato-neura* and *Paraphlebia*.

Note.—The wing figured by Prof. Needham (*Genealogic Study*, Pl. 53, fig. 4) as that of "*Philogenia* sp.?" is not *Philogenia*, but *Megapodagrion* Selys *sens. lat.* (*Allopodagrion* Foerster).

COMPARISON OF MEGAPODAGRION (NEBULOSUM²¹) WITH
PHENACOLESTES.

(*M. nebulosum* 2 ♂, Bolivia.)

These two forms agree in the characters above numbered 1, 3, 5, 7, 10, 11, 14, 16, 18, 21a, 22.

These two forms differ in the characters given below; after each number the difference shown by the *Megapodagrion* is stated.

2. Only two antenodals.
4. Stigma having its proximal edge less oblique.
6. The longitudinal veins show less curvature.
8. Between M_{1a} and M_2 at the wing-margin are two rows of cells.
9. M_2 separates from M_1 at .45 to .41 of the distance from nodus to stigma.
12. Between R_1 and M_3 at the wing-margin is only one row of cells.
13. Arculus at the second antenodal.
15. Between M_3 and M_4 is (a) only one cross-vein proximal to the level of the subnodus and (b) at the wing-margin are two rows of cells.
17. Between M_4 and Cu_1 are (a) 3-5 antenodal cells and (b) at the wing-margin only one row of cells.
19. Between Cu_1 and Cu_2 at the wing-margin is only one row of cells.
20. Area posterior to Cu_2 with only one row of cells.

²¹ *Megapodagrion nebulosum* Selys is referable to the recently proposed genus *Allopodagrion* of Förster, *Wien. Ent. Zeit.*, XXIX, p. 51, 1910.

21b. Anal vein separating from the hind margin of the wing at or slightly distad to the cubito-anal cross-vein.

Note.—The wing figured by Prof. Needham (*Genealogic Study*, pl. 53, fig. 4) as that of "*Philogenia* sp.?" is really that of a *Megapodagrion* Selys *sens. lat.*, *Allopodagrion* Foerster.

COMPARISON OF PARAPHLEBIA (ZOE AND ABROGATA) WITH
PHENACOLESTES.

(*P. zoe* ♂ ♀ Mexico, *P. abrogata* ♂ Guatemala.)

These two genera agree in the characters above numbered 4, 5, 10 (*abrogata* only), 12 (*abrogata* only), and 15b (*abrogata* only).

These two genera differ in the characters given below; after each number the difference shown by *Paraphlebia* is stated.

1. Nodus at one-fifth of the wing-length.
2. Only two antenodals.
3. Postnodals 40 ±.
6. Stronger caudal curvatures of the longitudinal veins generally as they approach the hind margin of the wing, although less strongly curved than in *Thaumaloneura*.
7. The one row of cells between M_1 and M_{1a} increases to 3 rows (*abrogata*) or 4–6 rows (*zoe*) at the wing-margin.
8. The one row of cells between M_{1a} and M_2 increases to 3 rows (*abrogata*) or 4–6 rows (*zoe*) at the wing-margin.
9. M_2 separates from M_1 at one-half (*abrogata*) or thirteen-thirtieths (= .43) of the distance from nodus to stigma (*zoe*).
10. Between M_2 and R_1 at the wing-margin are 3–6 rows of cells (*zoe*).
11. R_1 separates from M_{1+2} distad to the subnodus.
12. Between R_1 and M_3 at the wing-margin are 3–6 rows of cells (*zoe*).
13. Arculus at or even slightly distal to the second antenodal.
14. M_3 separating from M_{1+2} at or distad to the subnodus.
15. Between M_3 and M_4 (a) are no cross-veins proximal to the level of the subnodus (*zoe*, *abrogata*), and (b) at the wing-margin are 5–7 rows (front wings, *zoe*) or 6–11 rows (hind wings, *zoe*) of cells.
16. Quadrilateral with (a) its proximal and distal sides subparallel or slightly diverging caudad, and (b) its distal side hardly more than one and one-half times as long as the proximal side.
17. Between M_4 and Cu_1 is (a) one, or less than one, antenodal cell, and (b) at the wing-margin are 3 (*abrogata*) or 3–6 (*zoe*) rows of cells.
18. Cubito-anal cross-veins two, one between the levels of the first and second antenodals, but nearer to that of the first, the other nearly below the middle (or distal to the middle) of the quadrilateral.

19. Between Cu_1 and Cu_2 at the wing-margin are 10–14 rows of cells in *zoe*, seven (front wings) or five (hind wings) rows in *abrogata*.
20. Area posterior to Cu_2 less developed and with only two (*abrogata*, *zoe* ♀) or three (*zoe* ♂) rows of cells.²²
21. Anal vein separating from the hind wing-margin (a) distad to the level of the arculus and (b) nearly at the level of the second (distal) cubito-anal cross-vein.
22. Anal cross-veins, proximal to the level of the distal end of the quadrilateral, two (one in the front wings of the single female *zoe*).

COMPARISON OF DIMERAGRION N. GEN. (PERCUBITALE N. SP.) WITH PHENACOLESTES.

(*D. percubitale* 14 ♂, 6 ♀, British Guiana, Plate XIV, fig. 6.)

These two genera agree in the characters above numbered 1, 5, 7,²³ 8, 9, 12,²⁴ 16a, 16b.

These two genera differ in characters given below; after each number the difference shown by *Dimeragrion* is stated.

2. Two antenodals.²⁵
3. Postnodals 20–27 (front wing), 16–24 (hind wing), fewer in the females than in the males.
4. Stigma having its proximal edge more oblique.
6. The veins generally, posterior to M_1 , have a slighter caudal curvature, or are almost straight, as they approach the hind margin of the wing.
10. Between M_2 and R_1 is but one row of cells which at a distance of usually two²⁶ cells from the wing-margin increases to two rows.
11. R_1 separates from M_{1+2} at the first postnodal, or a little proximad thereto, or between the first and second postnodals.
13. Arculus at or very slightly distal to the second antenodal.
14. M_1 separates from M_{1+2} a little proximad to the subnodus, at least at seven-eighths' distance from arculus to subnodus.²⁷

²² The hind wings of some individuals, ♂ ♀, of *P. duodecima* have only one row of cells here.

²³ Two rows of cells between M_1 and M_{1a} for short distances proximad to the level of the stigma have occasionally been noted in *Dimeragrion*, viz.: for three cells in one front wing each of two males and for two cells in the other front wing of one of these same two males.

²⁴ Each of the three rows of cells in the terminal part of the area R_1 increases to two rows at the wing-margin in one front and one hind wing of the same male.

²⁵ A third antenodal placed between the usual two and confined to the costal space exists in the right hind wing only of one male.

²⁶ This increase to two rows has been noted as beginning at three cells from the margin in 2 front wings and 2 hind wings; at four cells in 3 front wings, 1 hind wing; at five cells in 2 front wings, 1 hind wing; at six cells in 2 front wings, 2 hind wings; at seven cells in 1 hind wing. All the individuals, seven in number, showing these variations are males.

²⁷ In one wing only it separated at the subnodus.

15. Between M_{1+2} and M_4 are (a) no cross-veins proximal to the level of the subnodus, and (b) between M_3 and M_4 is only one row of cells to the wing-margin.
17. Between M_4 and Cu_1 are (a) two to three antenodal cells (*sensu Selysii*) and (b) only one²⁸ row of cells to the wing-margin.
18. Cubito-anal cross-veins between the levels of the two antenodals varying from one to four, most frequently two; often an additional cross-vein proximal to the level of the first antenodal.²⁹
19. Between Cu_1 and Cu_2 is only one row of cells to the wing-margin.
20. Area posterior to Cu_2 with only one row of cells.
21. Anal vein separating from the hind wing-margin (a) distad to the level of the arculus and (b) distad to the level of the cubito-anal cross-vein or veins.
22. Anal cross-veins, proximal to the level of the distal end of the quadrilateral, one, situated a little proximad to the oblique vein which continues the distal side of the quadrilateral caudad, or at that oblique vein.

COMPARISON OF HETERAGRION (TRICELLULARE) WITH
PHENACOLESTES.

(*H. tricellulare* ♂, Guatemala.)³⁰

These two forms agree in the characters above numbered 5, 7, 8, 16, 18, 21a.

These two forms differ in characters given below; after each number the difference shown by the *Heteragrion* is stated.

1. Nodus at one-fourth of the wing-length.
2. Only two antenodals.
3. Postnodals 21-25.
4. Proximal edge of pterostigma more oblique, but the difference is less marked than in *Thaumatoneura*.
6. Longitudinal veins hardly curved at all caudad on approaching the wing-margin.
9. M_2 separates from M_1 at three-sevenths (= .43) of the distance from nodus to stigma.
10. Between M_2 and R_1 at the wing-margin is but one row of cells.
11. R_1 separates from M_{1+2} far distad (e.g., five to six cells) to the subnodus.
12. Between R_1 and M_3 at the wing-margin is but one row of cells.
13. Arculus at the second antenodal.
14. M_3 separating from M_{1+2} at or barely proximad to the subnodus.
15. Between M_3 and M_4 are (a) no cross-veins proximal to the level of the subnodus, as results from No. 14, and (b) at the wing-margin only one row of cells.

²⁸ Two rows exist in both hind wings of each of two females and in one hind wing of one male.

²⁹ For details see the specific description of *Dimeragrion percubitale*, *postea*, p. 269.

³⁰ The wing of *Heteragrion flavovittatum* has been figured by Needham (*General Study*, pl. 53, fig. 6).

17. Between M_4 and Cu_1 are (a) three antenodal cells³¹ and (b) at the wing-margin only one row of cells.
19. Between Cu_1 and Cu_2 at the wing-margin only one row of cells.
20. Area posterior to Cu_2 less developed, having only one row of cells.
- 21b. Anal vein separating from the hind wing-margin at or distad to the cubito-anal cross-vein.
22. Anal cross-vein as stated above for *Philogenia*.

COMPARISON OF MESAGRION, HETEROPODAGRION, AND NEURAGRION
WITH PHENACOLESTES.

Of three other American genera of the legion Podagrion, *Mesagrion* Selys, *Heteropodagrion* Selys and *Neuragrion* Karsch, each represented by a single species, the first from Bogota, the second from Quito, the third from Ecuador without more definite locality, I have seen no material.

According to the description of de Selys (1886), *Mesagrion* agrees with *Phenacolestes* in characters above numbered 3, 7 (probably), 8, 12, and 16a, and differs from *Phenacolestes* in the following characters:

2. Only two antenodal cross-veins.
10. Between M_2 and R_1 only one row of cells.
11. R_1 separates from M_{1+2} distad to the subnodus.
14. M_3 separates from M_{1+2} a little distad to the subnodus.
- 15b (Probably one row of cells between M_3 and M_4).
- 17a. Between M_4 and Cu_1 are two antenodal cells and b (probably one row of cells at the wing-margin).
18. Cubito-anal cross-veins ~~one~~, situated proximad to the level of the first antenodal cross-vein.
19. (Probably one row of cells between Cu_1 and Cu_2).
21. Anal vein separating from the hind margin of the wing (a) distad to the level of the arculus, and (b) distad to the level of the cubito-anal cross-vein.

According to de Selys' description (1886), *Heteropodagrion* agrees with *Phenacolestes* in characters above numbered 7 (probably), 10, 12, 14, and 16a, and differs from *Phenacolestes* in Nos. 2, 11, 15b (probably), 17a (and b probably), 18, and 21 in the same particulars as *Mesagrion* is above stated to differ from *Phenacolestes*. *Heteropodagrion* further differs from *Phenacolestes* in the following characters:

3. Postnodals 20-23.
8. (Probably one³² row of cells between M_{1a} and M_2 .)

³¹ Other species of *Heteragrion* have but two or one antenodal cell (Selys, 1886, pp. 54, 55).

³² Dr. Karsch, in his description of *Neuragrion*, states that it has "zwei Schaltsectoren . . . zwischen dem Nodal- und Ultranodalsector," and, further on, that it agrees in this respect with *Heteropodagrion*, but de Selys' description of the latter is silent on this point (although not for the same area of *Mesagrion*) and, I think, implies the absence of "Schaltsectoren" here.

According to Karsch's description,³³ *Neuragrion* agrees with *Phenacolestes* in characters above numbered 2 (but for the hind wings only),³⁴ 8, 10, 12, 14 (in so far as "Der Mediansector entspringt aus dem Principalis vor der vom Nodus absteigenden Ader"), and 16a, and differs from *Phenacolestes* in the following characters:

2. Only two antenodals on the *front* wings.
3. Postnodals 20-21.
11. R_1 separates from M_{1+2} near the first postnodal cross-vein.
13. Arculus distal to the normal second antenodal.
- 15b. Between M_3 and M_4 probably only one row of cells to margin.
- 17b. Between M_4 and Cu_1 probably only one row of cells to margin.
18. Cubito-anal cross-vein apparently proximal to ("vor," not "von"?) the level of the first antenodal.
19. Between Cu_1 and Cu_2 at wing-margin probably only one row of cells.
21. Anal vein separates from the hind wing-margin (a) at the level of the arculus and therefore (b) distad to the level of the cubito-anal cross-vein.

COMPARISON OF RHIPIDOLESTES (ACULEATA) WITH PHENACOLESTES.

(*Rhipidolestes aculeata* inhabits Formosa.)

According to the description and figure of Dr. Ris (1912), these two forms agree in the characters above numbered 1, 5, 6, 7, 8, 9, 10,³⁵ 12,³⁵ 15a, 16a (and perhaps 16b), 18, 19, and differ in the following characters possessed by *Rhipidolestes*:

2. Only two antenodals.
3. Postnodals about 22 (front wing) and 21 (hind wing).
4. Stigma, although having its proximal edge of about the same obliquity as in *Phenacolestes*, is shorter proximo-distally and its distal edge is less oblique.
11. R_1 separates from M_{1+2} proximad to the subnodus at very nearly three-fourths' distance from arculus to subnodus.
13. Arculus at the second antenodal.
14. M_2 separates from M_{1+2} at mid-way from arculus to subnodus.
- 15b. Between M_3 and M_4 at the wing-margin are three rows of cells.
17. Between M_4 and Cu_1 are (a) three antenodal cells and (b) at the wing-margin three rows of cells.
20. Area posterior to Cu_2 with only one row of cells.

³³ *Societas Entomologica* VI, p. 105, 1891. *Neuragrion* was described from a single female.

³⁴ "Im Hinterflügel drei Antecubitalqueradern, deren mittlere auf den Subcostalraum beschränkt ist."

³⁵ Each of the three rows in the terminal parts of these M_2 and R_4 areas may divide itself into two rows at two or three cells' distance from the wing-margin.

21. Anal vein separating from the hind wing-margin (*a*) distad to the level of the arculus and (*b*) distad to the level of the cubito-anal cross-vein.
22. Anal cross-vein at the vein descending from the distal end of the quadrilateral.

COMPARISON OF PODOPTERYX (ROSEONOTATUS) WITH
PHENACOLESTES.³⁶

(*P. roseonotatus* ♂ type of Selys (from Aru), 1 ♂ 1 ♀ Queensland determined by Mr. Herbert Campion; all three in the British Museum.)

These two genera agree in the characters above numbered 5, 7, 8,³⁷ 10,³⁸ 11,³⁹ 12⁴⁰, 16, 17*b*, and 18.⁴¹

These two genera differ in the characters given below; after each number the difference shown by *Podopteryx* is stated.

1. Nodus at one-fourth of the wing-length.
2. More often only two antenodals.⁴²
3. Postnodals 32-35.
4. Stigma less oblique, shorter in its proximo-distal dimension in proportion to its antero-posterior dimension.
6. The veins generally, posterior to M₁, have a more pronounced caudal curvature as they approach the hind margin of the wing.
9. M₂ separates from M₁ at .37 to .43 of the distance from nodus to stigma.
13. Arculus at the second antenodal.⁴³

³⁶ I am indebted to Mr. Herbert Campion for first calling my attention to the desirability of comparing this genus with *Phenacolestes*.

³⁷ There are 7-8 rows at the extreme margin, however.

³⁸ There are 5-7 rows at the extreme margin, however.

³⁹ In both front wings of the Queensland male and in the left front wing of the female R₄ is united with M₃ for from one-half to one cell distad of the subnodus.

⁴⁰ There are 5-6 rows at the extreme margin, however.

⁴¹ The right hind wing of the type male, the right front and left hind wings of the Queensland female have a second cubito-anal cross-vein proximal to the normal one and nearly mid-way between the levels of the first and second antenodals; the normal cubito-anal cross-vein is, in all, nearer to the level of the second antenodal. Prof. F. Foerster has also noted (Termesz. Fü. XXIII, p. 104, 1900) an instance of two cubito-anal cross-veins in *Podopteryx*.

⁴² The type male has three antenodals on all four wings, the third being distal to the other two and not reaching posteriorly beyond the subcosta. The two Queensland examples have only two antenodals on all the wings. The type male has also a subcostal cross-vein, proximal to the first antenodal, on both front wings and the right hind wing; this is lacking in the Queensland specimens.

⁴³ Slightly distal to the second antenodal on the two wings of the right side of the Queensland male. This is an appropriate place to record certain abnormalities of the type male, already mentioned by de Selys (1886); three of its wings have each a single medio-cubital cross-vein which, on the right front wing, is slightly proximal to the level of the normal first antenodal, but on the right hind wing and left front wing is slightly distal to the normal first antenodal. On the left hind wing there is merely a slight rudiment or vestige of such a cross-vein projecting forward as a stump from Cu slightly distad to the level of the first antenodal.

14. M_3 separates from M_{1+2} at .75 to .83 of the distance from arculus to subnodus.
15. Between M_3 and M_4 is (a) only one cross-vein proximal to the level of the subnodus and (b) one row of cells increasing to 9 to 11 rows at the wing-margin.
- 17a. Between M_4 and Cu_1 are 3+ to 5 antenodal cells (*sensu Selysi*).
19. Between Cu_1 and the *very much angulated* Cu_2 is one row of cells.
20. Area posterior to Cu_2 with many branches from that vein and four (front wings) or five (hind wings) rows of cells between it and the wing-margin.
21. Anal vein separates from the hind margin (a) at or slightly distad to the level of the arculus and therefore (b) much more distad to the level of the cubito-anal cross-vein.
22. More often no anal cross-veins.⁴⁴

COMPARISON OF ARGIOLESTES (ICTEROMELAS) WITH PHENACOLESTES.

(*A. icteromelas* ♂ ♀, Victoria, Australia.)

These two forms *agree* in the characters above numbered 1, 3, 5, 7, 8, 10, 11, 12, 14, 16, 18.

These two forms *differ* in the characters given below; after each number the difference shown by the *Argiolestes* is stated.

2. Only two antenodals.
4. Proximal edge of pterostigma nearly as in *Phenacolestes*, but the distal edge much more oblique.
6. Longitudinal veins posterior to M_1 with a stronger caudal curvature as they approach the margin.
9. M_2 separates from M_1 at four-tenths to one-half of the distance from nodus to stigma.
13. Arculus at or slightly distal to the second antenodal.
15. Between M_3 and M_4 is (a) one cross-vein proximal to the level of the subnodus, and (b) at the wing-margin are 8-10 (front wings) or 6-8 (hind wings) rows of cells.
17. Between M_4 and Cu_1 are (a) three (occasionally four) antenodal cells and (b) at the wing-margin are two to three rows of cells.
19. Between Cu_1 and Cu_2 at the wing-margin is only one row of cells.
20. Area posterior to Cu_2 with about two rows of cells.
21. Anal vein separating from the hind margin distad to the levels (a) of the arculus, and (b) of the cubito-anal cross-vein.
22. Anal cross-vein as stated above for *Philogenia*.

⁴⁴ There is an anal cross-vein proximal to the level of the distal end of the quadrilateral in the right front and right hind wings of the Queensland male and in the right front and left hind wings of the female. In the other eight wings, the first cross-vein in this part of the wing is at or slightly distal to the vein descending from the distal end of the quadrilateral and therefore should perhaps not be called an *anal* cross-vein, but a cross-vein posterior to Cu_2 . It has been marked Cu_{2a} by Mr. Williamson, 1913, p. 260.

COMPARISON OF CHLOROLESTES (FASCIATUS) WITH PHENACOLESTES.

(*Ch. fasciatus*, 2 ♂ Natal, 1 ♂ "Pirie Bush, S. Africa, A. N. Stenning 98-191"; all 3⁴⁵ in the British Museum.)

These two genera *agree* in the characters above numbered 5, 6, 7,⁴⁶ 8,⁴⁷ 9,⁴⁸ 10,⁴⁹ 15b, 16, 18,⁵⁰ and 22.

These two genera *differ* in the characters given below; after each number the difference shown by *Chlorolestes* is stated.

1. Nodus at four-fifteenths (= .27) of the wing-length.
2. Only two antenodals.⁵¹
3. Postnodals on the front wings 22-23, on the hind wings 17-20.
4. Stigma less oblique.
11. R₁ separates one to two cells distad to the subnodus, but in all cases nearer to M₃ than to M₁₊₂.
12. Between R₁ and M₃ is one row of cells which may increase to two rows at the extreme margin, symmetrically or asymmetrically.
13. Arculus at the second antenodal.
14. M₃ separating from M₁₊₂ at the subnodus.
- 15a. Between M₃ and M₄ are no cross-veins proximal to the level of the subnodus, the first cross-vein being very nearly at, but still slightly distal to, the level of the subnodus.
17. Between M₄ and Cu₁ are (a) two to three antenodal cells (*sensu Selysii*) and (b) one row of cells to the wing-margin.
19. Between Cu₁ and Cu₂ is one row of cells to the wing-margin.
20. Area posterior to Cu₂ with only one row of cells.
21. Anal vein separating from the hind wing-margin at, or slightly distal to, the level of the middle of the lower side of the quadrilateral, therefore far distad (a) to the level of the arculus and (b) to that of the cubito-anal cross-vein.

VARIOUS OLD WORLD GENERA AND PHENACOLESTES.

The genus *Wahnesia* Förster, of New Guinea, has the area posterior to Cu₂ fairly well developed, but not to the extent that it is in *Phenacolestes*, but as this genus differs from *Phenacolestes* in a number of characters, such as Nos. 2, 9, 11, 13, 14, 21, 22, a more detailed comparison has not been made.

⁴⁵ These three males have a brown band for the full width of the wing from the origin of M₂ distad to half-way from this point to the proximal end of the stigma.

⁴⁶ Occasionally increasing to two rows at the extreme margin.

⁴⁷ Each one of the three rows may divide, however, into two rows at the extreme margin.

⁴⁸ M₂ separates from M₁ at .28 to .33 of the distance from nodus to stigma.

⁴⁹ At the extreme margin there are 5-7 rows, however; but compare p. 229, *antea*.

⁵⁰ The cubito-anal cross-vein is nearer to the level of the first than to that of the second antenodal.

⁵¹ One of the Natal males has on the right hind wing an additional antenodal proximal to the normal first and confined to the costal area.

None of the following Old World genera seem to offer any close approximations to *Phenacolestes* and therefore have not been studied further in this connection: *Neurolestes* Selys, *Nesolestes* Selys, *Podolestes* Selys and *Synlestes* Selys. It may be noted, however, that in *Neurolestes trinervis* Selys, from Old Calabar, the three antenodals are so arranged that the arculus is at the third antenodal on all four wings.

GENERAL DISCUSSION OF THE VALUE OF THE VENATIONAL CHARACTERS COMPARED IN THE PRECEDING GENERA FOR THE DETERMINATION OF RELATIONSHIPS.

Many of the ideas which have been expressed on the changes which have taken place in the venation of Odonate wings have been based on the comparative morphology of living representatives of the order or on considerations of mechanical advantage. It does not follow that these foundations are trustworthy or that there has always been mechanical improvement in the wings. Degeneration is just as probable as progressive development. The actual course of phylogeny cannot be deduced from these considerations or from the data of morphology. It seems therefore desirable to attempt to ascertain what paleontological evidence exists affording clues to the descent of these insects, by tracing the modifications which the wings exhibit from the Carboniferous period down to the present time.

The characters of *Phenacolestes* and other genera which have been compared in the preceding pages under the numbers 1 to 22 may for the most part be arranged into two groups:

A. Those characters which are concerned with the positions of the points of separation of longitudinal veins and the positions and number of certain cross-veins.

B. Those characters which are concerned with the number of rows of cells existing between the longitudinal veins and their branches.

Group A includes numbers 1, 2, 3, 4, 9, 11, 13, 14, 15a, 16, 17a, 18, 21, and 22.

1. *The Position of the Nodus*.—The Protodonata of the Carboniferous and the Permian possessed no nodus; their subcosta (whose apex in the Odonata usually coincides with the nodus) reached often beyond mid-length of the wing, its minimal extent being to four-tenths of the wing-length in *Meganeurula* (Handlirsch⁵²). The earliest of

⁵² *Foss. Ins.*, p. 309, Taf. XXXI, figs. 37, 38.

the known Odonata, from the Lias, have been segregated by Handlirsch as a suborder, distinct from the existing Zygoptera and Anisoptera, under the name Anisozygoptera.⁵³ These insects usually possessed a nodus which lay at about mid-length of the wing. Handlirsch considers⁵⁴ that, of the recent forms, the Gomphidæ and Calopterygidæ have unquestionably the closest relations to the Anisozygoptera and are probably direct descendants thereof. The Agrioninæ of Selys have usually been looked on as descendants of Calopterygid(or -gine)-like forms.⁵⁵ The position of the nodus in Calopteryginæ and in Agrioninæ, respectively, has been stated as follows:

"The position of the nodus varies in both the Calopteryginæ and the Agrioninæ, but in general it is farther from the base of the wing in the former than in the latter. Among the first-named it is probably nearest the base (one-third the wing-length) in *Amphipteryx* and in *Chalcopteryx*,⁵⁶ among the second group it is farthest from the base (more than one-third the wing-length) in *Archilestes*. No Calopteryginæ have the nodus as near to the base as one-fifth the wing-length, but many Agrioninæ have it in that position."⁵⁷

This statement was based on conditions existing in recent forms. Very little is known of fossil Zygoptera of periods earlier than the Eocene⁵⁸ when *Dysagrion* and fragments named *Podagrion abortivum* by Scudder appear. From the Jurassic, Handlirsch lists⁵⁹ *Euphæopsis multinervis* Hagen, in fair preservation, and four possible but indecipherable species of *Pseudoeuphæa*, all considered to be allied to the living *Epallage*, *Euphæa*, etc., i.e., Calopteryginæ of Selys. *Euphæopsis multinervis* had the nodus at .44 of the wing-length. Another fossil of the same age is *Steleopteron deichmülleri* placed by Handlirsch in a separate family Steleopteridæ, which is considered to possess

⁵³ L.c., p. 463, August, 1906.

⁵⁴ L.c., p. 464.

⁵⁵ Calvert, *Trans. Amer. Ent. Soc.*, XX, pp. 211, 212, 1893; Ris, *Zool. Jahrb., Abth. Syst. Geog. Biol. Thiere*, IX, p. 623, 1896.

Cf. Needham, *Genealogic Study*, pp. 742, 748, 750, 1903; Handlirsch, *Foss. Ins.*, p. 472, 1906; Tillyard, *Proc. Linn. Soc. N. S. Wales*, XXXVI, p. 600, 1912; XXXVII, p. 406, 1913.

⁵⁶ In *Chalcopteryx* the nodus is at .37 (front wing) or .39 (hind wing) of the wing-length.

⁵⁷ Calvert, *Ent. Mo. Mag.*, (2), XIII, p. 31, 1902.

⁵⁸ Osborn, *The Age of Mammals*, 1910, p. 42, places the Green River shales in which these genera occur as equivalent to the Upper Ypresian, i.e., Middle Eocene.

⁵⁹ *Fossile Insekten*, pp. 595 et seq.

both Epallagid (Calopterygine) and Agrionine affinities. It had the nodus at only three-tenths of the wing-length.⁶⁰

No Cretaceous Zygoptera are listed by Handlirsch.

No ontogenetic data showing a proximal shifting in position of the nodus in any Zygopteron seem to have been published. If the evidence that may be drawn from the hypothetical descent of Agrioninæ (Selys) from Calopteryginæ (Selys) be left out of consideration, there yet appears to be good paleontological support, as sketched above, for the statement of Needham⁶¹ that shortening of the subcosta and retraction of the nodus toward the base of the wing is a developmental tendency away from the generalized condition of a long subcosta and a nodus remote from the wing-base. Yet it must be pointed that an upper Liassic fossil is known in which the subcosta ends at only .15 of the wing-length. This is *Protomyrmeleon brunonis* Geinitz, for which Handlirsch has erected the suborder Archi-Zygoptera and which he even suggests⁶² may have been a connecting link between the Protodonata and the Agrionidæ (= Agrioninæ Selys). Should such an ancestry for the latter group ever be demonstrated, the retraction of the nodus in living Odonata could not have the same significance, as a sign of specialization, which Needham has given it. The alternative suggestion of Handlirsch, that *Protomyrmeleon* is simply an analogue to the Agrionidæ (Agrioninæ Selys) whose descendants have become extinct, seems at present the more probable.

2. *The Number of Antenodal Cross-veins.*—*A priori* one might expect retraction of the nodus to be accompanied by a decrease in the number of antenodal cross-veins. No such proportional reduction exists, however. The two Calopterygine genera cited on p. 246 *anted* as having the most retracted nodus in that whole group, *Chalcopteryx*

⁶⁰ Still another Jurassic (Eichstädt) fossil is the *Agrion eichstättense* of Hagen, listed by Handlirsch as *Malmagrion eichstättense* (*Foss. Ins.*, p. 599). Hagen says of it (*Paleontographica*, X, p. 119, 1862): "Die Flügel sind 21 mm. lang, sehr schmal . . . Der Nodus liegt 6 mm. von der Basis. Das Geader ist nicht deutlich." His lithographic figure 5, Taf. XIV, agrees with these proportions.

Meunier (*Ann. Soc. Ent. France*, LXV, pl. 3, 1896) has given a figure labelled "Agrion? eichstättense Hagen type." It is photographic and represents an insect lying in the same position as that of Hagen's figure and of the same natural size. From Meunier's figure the position of the nodus cannot be recognized. In his text Meunier says, p. 131, "*Agrion eichstättense* Hagen. No. 150. Type de cet auteur. *Paleontographica*, t. x, p. 118 à 119, pl. XIV; Cassel, 1862." I cannot understand his expression a few lines farther in the same paragraph: "Cette échantillon a beaucoup d'analogie avec celui de Hagen."

⁶¹ *Genealogic Study*, p. 730.

⁶² *Foss. Ins.*, p. 472.

and *Amphiptyryx*, have respectively 23-26 (front wing), 21-23 (hind wing), and 7-10 (front wing), 7-8 (hind wing) antenodals. The paleontological evidence (with the exception of *Protomyrmeleon*, which has but one antenodal), including *Steleopteron*, is favorable to the view that reduction in the number of antenodals is a more recent acquisition. The preceding paper by Mr. Champion⁶³ discusses in a suggestive way the apparent evidence for such reduction afforded by recent Agrioninæ.

3. *The Number of Postnodal Cross-veins*.—Prof. Needham has concluded⁶⁴ that one of the developmental tendencies of the Odonata is: "Antenodal and postnodal cross-veins becoming reduced in number, stronger, more regular, sometimes matched in position or differentiated among themselves." In the Zygoptera this tendency is more evident in the antenodals than in the postnodals, the latter never becoming so few, absolutely, or relatively to the antenodals, as in certain Anisoptera, e.g., *Pachydiplax* (front wing: antenodals 6, postnodals 6) or *Miathyria simplex* (front wing: antenodals 7, postnodals 5).

In the Protodonata, owing to the great extension of the subcosta toward the apex of the wing, although there was no nodus, the potential postnodals were less numerous than the actual antenodals, assuming that the nodus of the Odonata developed at, or but little proximad to, the apex of the subcosta. With retraction of the nodus a greater number of postnodals in the costal space became possible. Numerous cases of subsequent reduction in the number doubtless occurred independently of each other, but the paleontological data are not sufficiently abundant to demonstrate progressive reduction in any one line of descent, however likely this may appear from the standpoint of mechanical advantage or of economy of vein-forming material.

4. *The Pterostigma*.—The stigma is an Odonate feature not possessed by the Protodonata. In those Anisozygoptera, which are considered by Handlirsch⁶⁵ to be ancestral to the Calopterygidae, viz., the Tarsophlebiidae, the stigma was fairly oblique and in one species at least braced at its proximal end.⁶⁶

In *Euphaeopsis*, of the Jurassic, the stigma is not oblique; in

⁶³ The Antenodal Reticulation of the Wings of Agrionine Dragonflies. This volume of these PROCEEDINGS, *anted*, pp. 220-224.

⁶⁴ *Genealogic Study*, p. 730.

⁶⁵ *Foss. Ins.*, p. 468.

⁶⁶ *Tarsophlebia eximia* of the Jurassic; Handlirsch, *l.c.*, Taf. XLVII, fig. 1.

Steleopteron, also of the same age, its proximal edge is not as oblique as in *Tarsophlebia eximia* and unbraced while its distal edge is more oblique. So far as one may judge from Handlirsch's figures⁶⁷ of Liassic Odonata the stigma was not oblique, or but moderately so, and unbraced; the forms in which it is preserved, however, seem to have but little relationship to the insects discussed in this paper. The paleontological evidence, therefore, furnishes little encouragement to use the stigma as a means of determining relationships.⁶⁸ It is important to notice, however, from the accompanying Table I, that the position of the stigma in the wing varies but little in different genera and species, so that it constitutes a relatively fixed landmark.

9, 11. *The Points of Separation of M_2 from M_1 and of R_1 from M_{1+2} .*—Prof. Needham has noted⁶⁹ a minor line of development within the Agrioninae, "in which nodus and quadrangle become more and more approximate, and the veins M_2 , R_1 , and M_3 migrate separately along vein M_1 from their accustomed places toward the stigma." This migration may be real or apparent. Retraction of the nodus with no change in position of the separation point of M_2 would result in a greater interval between nodus and M_2 and hence an apparent movement of the latter toward the stigma, but no real movement in respect to the wing as a whole. (Compare Table I, *Thaumato-neura*, *Rhipidolestes* and *Paraphlebia zoe* ♂.)

Real migration is where the separation-point of M_2 is transferred distad considered in relation to the total wing-length, and may or may not be accompanied by a change in the position of the nodus. (Compare Table I, *Perilestes* and *Dysagrion*, and *Perilestes*, *Argiolestes icteromelas* ♀ and *Melanagrion*.) These remarks will also apply to characters 11 (the separation-point of R_1 , including its bridge where it exists, from M_{1+2}), 14 (the separation-point of M_1 from M_{1+2}), 15a (the number of cross-veins proximal to the level of the subnodus between M_3 and M_4) and 17a (the number of antenodal cells between M_4 and Cu_1). In the preceding pp. 228-244, the statements for these characters have been made with reference, not to the wing-length, but to certain more or less varying landmarks, as the arculus, nodus, or stigma; this course has been in conformity with the established custom of descriptive writers.

⁶⁷ *L.c.*, Taf. XLII.

⁶⁸ *Cf.* Needham, *Genealogic Study*, pp. 710 at top and 730 at bottom.

⁶⁹ *L.c.*, p. 749.

TABLE I.

Positions of Certain Wing-details in Decimal Parts of Wing-length for Certain Odonata.

The measurements have been made from a single wing only in each species (front wing in living species).

GENUS AND SPECIES. (Recent, except where otherwise marked.)	Nodus. (1) ⁷⁰	Proximal costal angle of Stigma. (4)	Separation-point of M ₂ from M ₁ . (9)	Separation-point of R ₄ from M ₁ +2. (11)	Arculus. (13)	Separation-point of M ₃ from M ₁ +2. (14)	Distal anterior angle of quadrilateral. (16)	Separation-point of A from hind margin. (21)
<i>Protomyrmeleon brunonis</i> (Lias).....	.15	.88	.48	.21 (None)	.71	.53		
<i>Tarsophlebia eximia</i> (Jurassic).....	.45	.87	.47	.38	.14	.33	.15	
<i>Euphaeopsis multinervis</i> (Jurassic).....	.44	.83	.45	.3	.08	.15	.13	.02
<i>Steleopteron deichmülleri</i> (Jurassic).....	.30	.88	.40	.25	.10	.23	.15	.16
<i>Dysagrion packardii</i> (Eocene).....	.39	.87	.46	.39	.15	.30	.17	.07
<i>Phenacolestes mirandus</i> (Miocene)								
spmn. No. 3.....	.33	.83	.52	.35	.13	.29	.18	.10
<i>Phenacolestes mirandus</i> (Miocene)								
spmn. No. 5.....	.34	.84	.49	.34	.14	.26	.20	.11
<i>Thaumato-neura inopinata</i> ♂.....	.18	.90	.51	.20	.08	.17	.12	.06
" <i>pellucida</i> ♂.....	.18	.88	.51	.19	.08	.16	.12	.06
<i>Melanagrion umbratum</i> (Miocene).....	.29	.83	.48	.29	.16	.25	.27	.15
<i>Rhipidolestes aculeata</i> ♂.....	.29	.93	.51	.29	.18	.25	.22	.20
<i>Argiolestes icteromelas</i> ♂.....	.30	.89	.57	.31	.17	.28	.22	.18
" " ♀.....	.28	.89	.55	.32	.16	.28	.21	.18
<i>Allopodagrion nebulosum</i> ♂.....	.29	.90	.53	.31	.18	.27	.21	.16
<i>Lithagrion hyalinum</i> (Miocene).....	.34	.88	.50	.34	.20	.28	.24	.23
<i>Philogenia carrillica</i> ♂.....	.23	.91	.43	.26	.16	.23	.20	.18
" " ♀.....	.24	.90	.47	.26	.16	.24	.21	.20
" <i>terraba</i> ♂.....	.25	.91	.44	.26	.16	.23	.20	.19
<i>Paraphlebia zoe</i> ♂.....	.19	.93	.51	.24	.12	.20	.19	.17
" " ♀.....	.19	.95	.54	.23	.13	.20	.19	.18
" <i>abrogata</i> ♂.....	.20	.92	.55	.24	.14	.22	.22	.21
<i>Dimeragrion percubitale</i> ♂.....	.25	.81	.46	.28	.16	.25	.19	.18
<i>Heteragrion tricellulare</i> ♂.....	.23	.91	.50	.38	.16	.25	.20	.15
<i>Perilestes fragilis?</i> ♂.....	.28	.88	.66	.49	.21	.36	.25	.72
Summary:								
Maximum.....	.45	.95	.66	.49	.21	.36	.27	.23
Minimum.....	.15	.81	.40	.19	.08	.15	.12	.02
Range.....	.30	.14	.26	.30	.13	.21	.15	.21

⁷⁰ The number in parenthesis at the top of each vertical column is that under which that character has been treated throughout this paper.

⁷¹ In *Protomyrmeleon brunonis* M₂ separates from R₄.

⁷² A not developed as a vein separate from the hind margin.

The paleontological evidence with reference to the separation-points of M_2 , R_1 and M_3 is that the Liassic fossils (with the exception of *Protomyrmeleon*) had M_2 separating at the subnodus, and R_1 (including its bridge) and M_3 proximad to the nodus;⁷³ none of these fossils which have the nodal region preserved are considered by Handlirsch to be ancestral to Zygoptera, however. For many of the Jurassic remains⁷⁴ the same statement as to the positions of these separation-points holds true, including *Tarsophlebia eximia* and *Euphæopsis multinervis*; *Steleopteron* had R_1 and M_3 arising well proximad to the nodus, M_2 only a short distance distad to the nodus; *Palaephlebia synlestoides*, which Brauer, Redtenbacher and Ganglbauer assigned to the legion Podagrion of Selys⁷⁵ but which Handlirsch regards as an Anisozygopter of doubtful position,⁷⁶ is imperfectly preserved, but seems to have had the origins of both R_1 and M_2 distinctly distal to the nodus, while that of M_3 was probably proximal to the nodus. *Palaephlebia* came from the Dogger, or lower Jurassic, of Siberia, and is therefore older than the other Jurassic fossils just referred to, all of which are Malm or Upper Jura. The paleontological evidence is consequently not altogether satisfactory and at least admits of the possibility of the separation-points of these three veins having fluctuated proximad or distad from time to time. Prof. Needham's statement as to their migration, quoted above, has not that evidence from other sources than comparative morphology which is necessary to enable us to use these characters with perfect confidence.

13. *The position of the arculus* has been relatively stable, so that there is little reason for attaching any special phylogenetic value to it.

14. *The Point of Separation of M_3 from M_{1+2} .*—(See the discussion above under Nos. 9, 11.)

15a. *The Number of Cross-veins, Proximal to the Level of the Subnodus, between M_3 and M_4 .*—This character is closely dependent on No. 14, for the more proximal is the separation-point of M_3 from M_{1+2} , the greater is the area available for these cross-veins. In the genera here compared the greatest number of cross-veins, two, is found in *Phenacolestes*, *Lithagrion*, and *Melanagrion*, all of which have a more proximal origin of M_3 . The reverse case is not necessarily true, as *Philogenia*, *Allopodagrion*, and *Dysagrion*, with an equally proximal

⁷³ Handlirsch, *Foss. Ins.*, Taf. XLII.

⁷⁴ *L.c.*, Taf. XLVII.

⁷⁵ *Mem. Acad. Imp. Sci. St. Petersb.*, (7), XXXVI, p. 6, 1889.

⁷⁶ *Foss. Ins.*, p. 584.

origin of M_3 (see Table I), have but one such cross-vein. *Tarsophlebia eximia* had five in its hind wing (these veins are not preserved in the front wing) and *Steleopteron* had two.

16. *The Obliquity of the Quadrilateral.*—The shape of the quadrilateral becomes more oblique with the greater divergence of its proximal and distal sides. Prof. Needham has assumed⁷⁷ that a nearly rectangular quadrilateral, in which opposite sides are parallel to each other, is the more primitive form and that from it at least two kinds of oblique quadrilaterals have arisen. There seems to be no published ontogenetic evidence in favor of this view and the only paleontological evidence not contradicting it is limited to that puzzling Liassic form, *Protomyrmeleon*, in which, if Handlirsch's interpretation of the venation be correct,⁷⁸ R and M are still distinct from the base distad, and there is no trace of a beginning arculus.

On the other hand, all the Jurassic fossils, which are not clearly Anisoptera, show an *oblique* quadrilateral with its posterior distal angle acute.⁷⁹ This holds true not only for those fossils referred by Handlirsch to the Zygoptera like *Euphæopsis* and *Steleopteron*, or for forms regarded by him as ancestral to Zygoptera, as *Tarsophlebia* (and including a Liassic fragment, *T. westwoodi*⁸⁰), but also for other Anisozygoptera not apparently forefathers of the Zygoptera, as *Stenophlebia*, *Isophlebia*, and *Anisophlebia*. *Dysagrion* of the Eocene had an oblique quadrilateral, and in fact not one of the published descriptions and figures of Tertiary Zygoptera listed by Handlirsch⁸¹ shows a rectangular quadrilateral; all have it oblique.

It therefore seems necessary to regard the oblique quadrilateral as the more primitive and the rectangular as a later appearance.

⁷⁷ *Genealogic Study*, p. 717, fig. 11, p. 731. See also Williamson, 1913, p. 259, and figs. 1 and 2 on p. 260.

⁷⁸ *Foss. Ins.*, Taf. XLII, fig. 14.

⁷⁹ *L.c.*, Taf. XLVII. Hagen, indeed (*Paleontographica*, X, Taf. VIII, f. 8), shows the quadrilateral of the Jurassic *Euphæa longiventris* as rectangular. In his description, p. 123, he says, "Noch undeutlicher ist der übrige Theil des Geäders . . . das Viereck ist gleichfalls undeutlich," and he gives no details of its shape. Meunier's photographic reproductions (*Arch. Mus. Teyler*, 2, VI, pl. XXVI, figs. 81, 82, cf. p. 127), not of Hagen's type, but of other specimens in the Munich Museum, show the shape of the quadrilateral as indecipherable. Handlirsch (*Fossil Ins.*, p. 580) places *Euphæa longiventris* Hagen as a synonym of *Tarsophlebia eximia* Hagen and remarks, "Bezüglich Hagens *Euphæa longiventris* [longiventris] habe ich zu bemerken, dass der von ihm t. 13 f. 8 abgebildete Flügel allerdings von jenem der *Tarsophlebia* wesentlich abweicht. Aus der Beschreibung ergibt sich jedoch, dass die basale Partie des Flügels an dem Fossile kaum zu entziffern ist, so dass wir diese Reconstruction als werthlos betrachten können."

⁸⁰ *Foss. Ins.*, Taf. XLII, fig. 6.

⁸¹ *L.c.*, pp. 896-899, 1357.

17a. *The Number of Antenodal Cells between M_1 and Cu_1 .*—This character is in general dependent on the degree of retraction of the nodus, as the more the nodus approaches the base of the wing the smaller is the area available for these cells. This correlation, although not absolute and exact in the genera here compared, holds in a rough way. We may therefore regard reduction in the number of antenodal cells as a specialization. There appear to have been 12 on the front wing, 13 on the hind wing of *Tarsophlebia eximia* (counting only a single row—there are two rows in part of this area), almost three in *Steleopteron*. A reduction in the number of antenodal cells may occur independently of retraction of the nodus, however, by the greater development of the quadrilateral distad. (Compare Table I, *Thaumatonera* and *Paraphlebia*, also pp. 233, 237 *anted.*)

18. *The Number of Cubito-anal Cross-veins.*—These veins are usually small and weak in the Zygoptera, and they have not been preserved well in the fossils, so that few data on their development in point of geologic time are available. Perhaps the same kind of evidence which Mr. Champion has secured⁸² for the reduction of the antenodals might be obtained for these cross-veins also. It is conceivable that the ancestral Zygopteron might have had a moderate number and that some of its descendants might have acquired more, others fewer. While recognizing the paucity of evidence, I am inclined to view reduction in their number as specialization, in harmony with the reduction of antenodals.

21. *Petiolation of the Wing.*—When the anal vein (A) separates from the hind margin of the wing at some distance from the base, a stalk-like appearance of the wing-base is produced, especially on the hind margin, to which the term “pétiolée” was long ago applied by de Selys. Petiolation involves a reduction of the anal area and is specifically mentioned by Prof. Needham⁸³ as a fundamental developmental tendency in the Agrionidæ (= Agrioninæ Selys). It is not visible in *Protomyrmeleon* (Lias); in *Tarsophlebia*, *Stenophlebia*, *Isophlebia*, or *Anisophlebia* (Jura). It seems to have been well-marked in *Steleopteron*, while the shape of the wing-outline certainly suggests it in *Euphæopsis*, although here A may have remained distinct from the hind margin.⁸⁴ It seems justifiable to assume that the farther distad petiolation has progressed, the more specialized the insect.

⁸² These PROCEEDINGS, p. 223, *anted.*

⁸³ *Genealogic Study*, p. 748. Cf. Ris, 1912, p. 45; Tillyard, 1913, p. 444.

⁸⁴ Handlirsch, *Foss. Ins.*, Taf. XLVII.

22. *The Number of Anal Cross-veins.*—Progressive petiolation reduces the area available for anal cross-veins, but not always to the extent one might expect. Thus of American genera discussed in this paper which have the petiolation extending distad to the arculus, *Paraphlebia* has 2, *Philogenia* 0, *Dimeragrion* 1 or 0, *Lithagrion* 0 anal cross-veins. Calopteryginæ (Selys) have on the whole more anal cross-veins than do the Agrioninæ Selys. If the latter are descendants of some of the former and if *Tarsophlebia* stands in an ancestral position to the Zygoptera, then we may consider reduction in number of the anal cross-veins as a specialization. *Protomyrmeleon*, *Tarsophlebia*, *Isophlebia*, and *Anisophlebia* each had not less than four anal cross-veins, some of them many more. In *Euphæopsis* they are not preserved, in *Steleopteron* they appear to have been present only distad to the level of the outer (distal) end of the quadrilateral.

B. The characters included under group B on p. 245, *anted*, are Nos. 7, 8, 10, 12, 15b, 17b, 19, and 20. In the comparisons made in the early pages of this paper, these characters have been stated in terms of numbers of rows of cells found between the branches of the great veins. They may be stated also by specifying the number of interposed, or supplementary, sectors in each area, and have usually been so stated in the taxonomic literature. It is easy to translate one method of statement into the other, as one row of cells implies no supplementary sector, two rows of cells one supplementary sector, three rows two sectors, etc. The individual variation to which they are subject increases as the hind margin of the wing is approached.

The Protodonate wing of the Carboniferous and the Permian was richly veined with many supplementary sectors. So also were the Odonate wings of the Lias, excepting *Protomyrmeleon*; if Handlirsch's interpretation of its venation be correct,⁸⁵ there was but one row of cells between each of the branches of the main veins, except between R_1 and M_2 (two rows increasing to three), M_2 and M_4 (two rows), M_4 and Cu_1 (two rows), and posterior to A (two rows).

Turning to the Odonata of the Jura,⁸⁶ we find richly veined wings in the Anisozygoptera (*Tarsophlebia*, *Stenophlebia*, *Isophlebia*, *Anisophlebia*), and in *Euphæopsis*; *Steleopteron* has one row of cells between M_1 and M_{1a} , M_{1a} and M_2 , M_2 and R_1 , M_3 and M_4 , and between the veins posterior to Cu_1 , three rows between R_1 and M_2 , two rows between M_4 and Cu_1 .

⁸⁵ *Foss. Ins.*, Taf. XLII, fig. 14.

⁸⁶ Handlirsch, *Foss. Ins.*, Taf. XLVII.

If Handlirsch be correct in his conjecture⁸⁷ that the Protodonata have descended from such genera of the Dictyoneuridæ (Palæodictyoptera) as *Stenodictya*, the dense venation of the former evolved from the equally dense, but more irregular, network of the latter. The Odonata then carried reduction in the network to a greater degree than in the Protodonata and reduction in the rows of cells of the areas now under discussion would, therefore, be a specialization. We must then recognize that as early as the Lias such reduction was carried in *Protomyrmeleon* to a greater degree than is shown by many living genera and a similar statement must be made for *Steleopteron* of the Jura. That any of these reduced venations were capable of giving rise to more exuberantly veined descendants would be denied by many phylogenists, but the actual proof of such a denial is not at hand for the Odonata. We have, therefore, only the general tendency to guide us when we assume that reduction in density of venation in the characters of group B means specialization. On the other hand, we must suppose that such richly veined forms of the present day as *Thaumatoneura*, *Calopteryx*, *Thore*, and *Neurothemis* have either preserved the dense venation of, perhaps Mesozoic, ancestors or that they have acquired their density by hypertrophy in the course of generations.⁸⁸ Here, as elsewhere in this discussion, the possibility of further light from ontogenetic studies is very evident.

Summarizing the results of this discussion, we conclude that later phylogenetic status is denoted by the following conditions in the characters of the genera compared in this paper:

1. Retraction of the nodus toward the wing-base.
2. Reduction in the number of antenodals.
- (3. Reduction in the number of postnodals.)
- (7. Reduction in the number of rows of cells between M_1 and M_{1a} .)
- (8. Reduction in the number of rows of cells between M_{1a} and M_2 .)
9. More distal position of origin of M_2 .
- (10. Reduction in the number of rows of cells between M_2 and R_1 .)
11. More distal position of origin of R_1 .
- (12. Reduction in the number of rows of cells between R_1 and M_3 .)
14. More distal position of origin of M_3 .
- 15a. Reduction in number of cross-veins proximal to the subnodus between M_3 and M_4 .

⁸⁷ *L.c.*, p. 305.

⁸⁸ The latter alternative is apparently that held by Dr. Ris for the analogous cases of *Pantala* and *Tramea*. 1912, p. 46.

- (15b. Reduction in the number of rows of cells between M_3 and M_4 .)
- 16. Rectangular quadrilateral.
- 17a. Reduction in the number of antenodal cells between M_4 and Cu_1 .
- (17b. Reduction in the number of rows of cells between M_4 and Cu_1 .)
- (18. Reduction in the number of cubito-anal cross-veins.)
- (19. Reduction in the number of rows of cells between Cu_1 and Cu_2 .)
- (20. Reduction in the number of rows of cells posterior to Cu_2 .)
- 21. More distal petiolation of the wing.
- (22. Reduction in the number of anal cross-veins.)

I have endeavored to indicate that the paleontological evidence is not as strong for some of these statements, by enclosing them in parentheses, as for those left unenclosed. There is no ontogenetic evidence as yet.

These conclusions and the data on which they are based merely permit us to determine the probable course, or direction, of change in certain features of the venation. They do not enable us to decide as to the relative order in which the different features changed, whether, for example in the ancestors of living Podagrionine genera, M_3 assumed a position at or distad to the subnodus at an earlier, or at a later, period than the reduction of the number of rows of cells in the area posterior to Cu_2 occurred, although, given a character, we can say which genera should come first and which should follow in our phylogenetic list in respect to the modifications of that character. In the imperfection of our knowledge, we are largely guided, therefore, by motives of convenience in selecting the primary characters on which a classification of the members of this group is to be based. Having regard to all these considerations, the following scheme for a phylogenetic grouping of the genera of Selys' legion Podagrion is presented.

SYNOPSIS OF VENATIONAL CHARACTERS OF GENERA OF THE LEGION PODAGRION.

Legion Podagrion Selys 1862.

M_3 separating from M_{1+2} nearer to the nodus than to the arculus. Pterostigma normal, *i.e.*, consisting of but a single cell. Cu_2 present. Supplementary sectors present, at least between M_{1+2} and M_2 , except in *Perilestes*.

A.— M_3 separating from M_{1+2} proximad to the subnodus, at six-tenths or more of the distance from arculus to subnodus (half-way in *Rhipidolestes*).

- B.—Antenodals more than two on all the wings, or on the hind wings only (*Neuragrion*). (See also the parenthesis following rubric BB.)
- C.—Area posterior to Cu_2 with three or more rows of cells, A separating from the hind margin proximad to the level of the arculus, R_s beginning at the subnodus.
- D.—Quadrilateral oblique at distal end.
- E.—Supplementary sectors between M_1 and Cu_1 absent; between M_{1a} and M_2 five, between R_s and M_3 eight rows of cells, respectively. *Dysagrion* Scudder, 1878 (Eocene, Wyoming).
- EE.—Supplementary sectors between M_1 and Cu_1 three or four; between M_{1a} and M_2 three, between R_s and M_3 three rows of cells respectively. *Phenacolestes* Cockerell, 1906 (Miocene, Colorado).
- DD.—Quadrilateral almost rectangular at both proximal and distal ends; many supplementary sectors from M_1 to hind margin. *Thaumatoneura* M'Lachlan, 1897 (Central America).
- CC.—Area posterior to Cu_2 with only one row of cells, A separating from hind margin at or distad to the level of the arculus.
- F.— R_s beginning at subnodus, supplementary sectors from M_{1a} to M_1 , two or more cubito-anal cross-veins. *Neurolestes* Selys, 1882 (West Africa).
- FF.— R_s beginning distad to the subnodus, supplementary sectors from M_{1a} to M_3 , one cubito-anal cross-vein. *Neuragrion* Karsch, 1891 (Ecuador).
- BB.—Antenodals two only (more than two have been noted in occasional specimens of *Podopteryx*, a genus included under this rubric).
- G.— R_s beginning proximad to the subnodus.
- H.—Area posterior to Cu_2 with two rows of cells, A separating from the hind margin proximad to the level of the arculus, supplementary sectors from M_{1a} to M_1 . *Melanagrion* Cockerell, 1907 (Miocene, Colorado).
- HH.—Area posterior to Cu_2 with one row of cells, A separating from hind margin distad to the level of the arculus, supplementary sectors from M_{1a} to Cu_2 . *Rhipidolestes* Ris, 1912 (Formosa).
- GG.— R_s beginning at the subnodus.
- J.—Area posterior to Cu_2 with 4–5 rows of cells; A separating from the hind margin at or distad to the level of the arculus, supplementary sectors from M_{1a} to Cu_1 . *Podopteryx* Selys, 1871 (Aru, Queensland).
- JJ.—Area posterior to Cu_2 with 2 rows of cells, otherwise as in *Podopteryx*. *Argiolestes* Selys, 1862, sens. strict.⁸⁹ (Australian region).

⁸⁹ As here limited includes only the groups 1C and 2 of Selys' Revision of 1886, p. 81. See the remarks of Martin on the variability of the points of departure of M_3 and R_s in *Argiolestes*, *Boll. Soc. Ent. Ital.*, ix, p. 201, 1909.

- JJJ.—Area posterior to Cu_2 with one row of cells.
- K.—Old World Genera.⁹⁰
- L.—A separating from the hind margin proximad to the level of the arculus (some *Podolestes* fall here—see below under OO).
- M.—Supplementary sectors from M_1 to M_3 and between M_4 and Cu_2 , none between M_3 and M_4
Rhinagrion nom. n. for *Amphilestes* Selys, 1862⁹¹ (Malaysia).
- MM.—Supplementary sectors (two each) from M_{1a} to R_1 , one between M_3 and M_4
Mesopodagrion McLachlan, 1896 (Thibet, West China).
- LL.—A separating from the hind margin distad to the level of the arculus.
- N.—Quadrilateral with its anterior side more than half as long as its posterior side, Cu_1 only slightly arched forward on leaving the quadrilateral.
- O.—Arculus distinctly distal to the second antenodal, supplementary sectors (two each) from M_{1a} to M_4*Nesolestes* Selys, 1891 (Madagascar).
- OO.—Arculus at, or but slightly distal to, the second antenodal, supplementary sectors (one or two each) from M_{1a} to M_4
Podolestes Selys, 1862 (Labuan, Sumatra).
- NN.—Quadrilateral with its anterior side half, or less than half, as long as the posterior side; Cu_1 strongly arched forward on leaving the quadrilateral, supplementary sectors (one each) from M_1 or M_{1a} to R_1 or M_3
Synlestes Selys, 1869 (Australia).
- KK.—New World Genera.
- P.—A separating from the hind margin proximad to the level of the arculus, supplementary sectors from M_{1a} to R_1 and between M_3 and M_4 .
- Q.—Area M_{1a} to M_2 with three rows of cells, two unbroken supplementary sectors between M_3 and R_1
Megapodagrion Selys, 1885, sens. strict. (Brazil).
- QQ.—Area M_{1a} to M_2 with two rows of cells, one zigzag supplementary sector between M_3 and R_1
Allopodagrion Foerster, 1910 (Northern South America).

⁹⁰ Although the first two subdivisions, K and KK, under rubric JJJ are geographical, not structural, for convenience' sake, the structural features under K and KK are sufficiently detailed to enable the identification of a form of unknown habitat to be made.

⁹¹ The name *Amphilestes* Selys is preoccupied by *Amphilestes* Owen (*Encyc. Brit.*, 8th edit., XVII, p. 157, 1859) for a fossil mammal. *Rhinagrion* is suggested by de Selys' emphasis of one of the characters of his *Amphilestes* as "Tête robuste, à épistome saillant."

- PP.—A separating from the hind margin distad to the level of the arculus.
- R.—Nodus more nearly at one-third of the wing-length, no supplementary sectors between R₁ and M₁..... *Lithagrion* Scudder, 1882 (Miocene, Colorado).
- RR.—Nodus more nearly at one-fourth of the wing-length, at least two supplementary sectors between R₁ and M₁. (Supplementary sectors from M_{1a} to M₁ or Cu₁)..... *Philogenia* Selys, 1862, except *P. terraba* Calv. (Costa Rica to Brazil).
- GGG.—R₁ beginning distad to the subnodus, A separating from the hind margin distad to the level of the arculus.
- S.—Area posterior to Cu₂ with usually two or more rows of cells, supplementary sectors from M₁ to Cu₂ or to hind margin, two cubito-anal cross-veins, the second under the quadrilateral..... *Paraphlebia hyalina* Brauer, 1871 (Mexico).
- SS.—Area posterior to Cu₂ with one row of cells, no supplementary sectors posterior to M₁.
- T.—Supplementary sectors from M_{1a} to M₁, one cubito-anal cross-vein.
- U.—Antenodal cells between M₁ and Cu₁ two or three (otherwise as above under RR)..... *Philogenia terraba*²² Calvert, 1907 (Costa Rica).
- UU.—Antenodal cells between M₁ and Cu₁ one; quadrilateral very long, reaching to the level of the nodus..... *Allolestes* Selys, 1869 (Seychelles).
- TT.—Supplementary sectors from M_{1a} to M₁ (often none between M₂ and R₁), usually more than one cubito-anal cross-vein..... *Dimeragrion* new genus (Guiana).²³
- TTT.—Supplementary sectors between M₂ and R₁ and (two long ones) between R₁ and M₁, one cubito-anal cross-vein..... *Heteropodagrion* Selys, 1885 (Ecuador).

²² In spite of the fact that *Philogenia terraba* falls here, instead of under RR with the rest of the genus *Philogenia*, the only difference which separates it is the slight one of the more distal position of the separation-point of R₁ from M₁+₂, and I think this does not entitle *terraba* to a different generic name.

²³ The genus *Neuragrion* Karsch was described from a single female and has been placed under rubric FF above. If additional specimens should prove to have only two antenodals, the genus would fall under TT and would differ from *Dimeragrion* presumably, at least, in possessing only one cubito-anal cross-vein.

- TTTT.—Supplementary sector one between M_{1a} and M_2 , one cubito-anal cross-vein, quadrilateral not reaching the level of the nodus.....
Protolestes Foerster, 1899 (Madagascar).
- AA.— M_2 separating from M_{1+2} at the subnodus, only two antenodals, R_1 beginning distad to subnodus.
- V.—Area posterior to Cu_2 with usually two or more⁹⁴ rows of cells, A separating from the hind margin distad to the level of the arculus.
- W.—Supplementary sectors from M_1 to Cu_2 or to hind margin; two cubito-anal cross-veins, the second under the quadrilateral.....*Paraphlebia* Hagen, 1861 (Mexico, Guatemala).
- WW.—Supplementary sectors from M_{1a} to Cu_1 or to hind margin, one cubito-anal cross-vein.....*Wahnesia* Foerster, 1900 (New Guinea)⁹⁵
- VV.—Area posterior to Cu_2 with one row of cells, one cubito-anal cross-vein.
- X.—Supplementary sectors from M_{1a} to M_1 , A separating from hind margin at or distad to arculus.....
Chlorolestes Selys, 1862 (South Africa).
- XX.—Supplementary sectors confined to area M_{1a} to M_2 , A separating from hind margin proximad to, at, or distad to, the level of the arculus.....
Heteragrion Selys, 1862 (Mexico to Argentina).⁹⁶
- AAA.— M_2 separating from M_{1+2} distad to the subnodus, only two antenodals, R_1 beginning distad to the subnodus, A separating from the hind margin distad to the level of the arculus (or not developed as separate from the hind margin in *Perilestes*).
- Y.—Area posterior to Cu_2 with two or three rows of cells.
- Z.—Supplementary sectors from M_1 to Cu_2 or to hind margin, other characters as above under W.....
Paraphlebia (see W).

⁹⁴ Both sexes of *Paraphlebia duodecima* Calv. have only one row of cells posterior to Cu_2 on the hind wings, but two or more rows on the front wings.

⁹⁵ This is group 1B of *Argiolestes* in Selys' Revision of 1886, p. 81.

⁹⁶ In his Synopsis of 1862, p. 31, de Selys suggested the separation of *H. petiolatum* as a separate subgenus under the name of *Oxystigma*, chiefly distinguished from *Heteragrion* by the long quadrilateral reaching almost to the level of the nodus, the pterostigma extremely pointed proximally and only one antenodal cell (between M_1 and Cu_1), but he did not adopt this suggestion in his Revision of 1886. The name *Oxystigma* does not appear in Kirby's Catalogue.

- ZZ.—Supplementary sectors from M_{1a} to Cu_1
Metagrion n. gen.⁷ (New Guinea).
 YY.—Area posterior to Cu_2 with one row of cells, no supplementary sector posterior to M_3 .
 a.—Quadrilateral not touching the hind margin of the wing, supplementary sectors between M_{1a} and M_2 and between R_1 and M_3
Mesagrion Selys, 1885 (Colombia).
 aa.—Quadrilateral touching the hind margin of the wing, no supplementary sectors except a rudiment between M_2 and R_1 or between R_1 and M_3 , arculus well distal to the second antenodal.....
Perilestes Selys, 1862
 (Costa Rica, Guiana, Brazil).

In view of the observations on the imperfection of our knowledge, made on p. 256, in introducing the preceding Synopsis, it seems useful to offer also this

OUTLINE OF AN ALTERNATIVE GROUPING OF THE GENERA OF THE LEGION PODAGRION.

- A.—Area posterior to Cu_2 with more than one row of cells.
 B.—A separating from the hind margin proximad to the level of the arculus, M_3 separating from M_{1+2} proximad to the subnodus.
 C.— R_1 beginning proximad to the subnodus, two antenodals, supplementary sectors M_{1a} to M_4 , two rows of cells posterior to Cu_2*Melanagrion*.
 CC.— R_1 beginning at the subnodus, more than two antenodals.....*Dysagrion*, *Phenacolestes*, *Thaumatonera*.
 BB.—A separating from the hind margin at, or distad to, the level of the arculus, normally two antenodals.
 D.— M_3 separating from M_{1+2} proximad to the subnodus.
 E.— R_1 beginning at the subnodus.....*Podopteryx*, *Argiolestes*.
 EE.— R_1 beginning distad to the subnodus.....*Paraphlebia hyalina*.
 DD.— M_3 separating from M_{1+2} at, or distad to, the level of the subnodus.....*Paraphlebia*, *Wahnesia*, *Metagrion*.
 AA.—Area posterior to Cu_2 with only one row of cells.
 F.—A separating from the hind margin proximad to the level of the arculus.

⁷ Type: *Argiolestes postnodalis* Selys. This genus is group 1A of Selys' Revision of 1886, p. 81.

- G.— M_3 separating from M_{1+2} proximad to the subnodus, R_1 at the subnodus.....
Rhinagrion, Mesopodagrion, Megapodagrion, Allopodagrion, Podolestes.
- GG.— M_3 separating from M_{1+2} at the subnodus, R_1 distad to the subnodus.....*Heteragrion.*
- FF.—A separating from the hind margin at, or distad to, the level of the arculus (or not developed as a vein distinct from the hind margin in *Perilestes*).
- H.— M_3 separating from M_{1+2} proximad to the subnodus.
- J.— R_1 beginning proximad to subnodus.....
Rhipidolestes.
- JJ.— R_1 beginning at the subnodus.....
Neurolestes (3 antenodals), *Lithagrion, Philogenia, Nesolestes, Podolestes, Synlestes.*
- JJJ.— R_1 beginning distad to the subnodus.....
Neuragrion (3 antenodals on hind wings), *Philogenia terraba, Allolestes, Dimeragrion, Heteropodagrion, Protolestes.*
- HH.— M_3 separating from M_{1+2} at the subnodus.....
Chlorolestes, Heteragrion.
- HHH.— M_3 separating from M_{1+2} distad to the subnodus.....*Mesagrion, Perilestes.*

The preceding Synopsis, pp. 256-261, will enable one using this Alternative Grouping to distinguish from each other the genera listed under each final rubric.

A comparison of Synopsis and Alternative Grouping will show that, in spite of a number of differences in the order in which the genera appear in the two schemes, both begin with and both end with nearly the same forms, which may therefore be regarded as, respectively, the oldest and the youngest members, phylogenetically, of the legion Podagrion.

THE RELATIONSHIPS OF PHENACOLESTES.

If the conclusions reached on pp. 255-256 be correct,

Dysagrion of the Eocene of Wyoming, the oldest member of the legion Podagrion now known, cannot have been an ancestor of any of the three known Miocene genera from Colorado, *Phenacolestes, Lithagrion*, and *Melanagrion*, because it lacked supplementary sectors between M_1 and Cu_1 . *Melanagrion* indeed possesses one more primitive feature than is found in any of the other three of these extinct genera, viz., R_1 begins proximad to the subnodus.

Phenacolestes has preserved more primitive characters than has

its contemporary *Lithagrion*, as may be seen by a comparison of those numbered 2, (4?), 12, 15b, 17a and b, 19, 20, 21a and b, and 22, on pp. 227 and 232, *anted.*

Phenacolestes is more primitive than its contemporary *Melanagrion* in characters Nos. 1, 2, 12, 15b, 17a and b, 19, 20, 21b and 22, but less primitive in characters Nos. 3, (4?), and 11. See pp. 227 and 231, *anted.*

Phenacolestes may have been the ancestor of the New World genera *Megapodagrion*, *Allopodagrion*, and *Perilestes* and of the African *Chlorolestes*, unless it shall be shown that the less oblique pterostigma possessed by all four of these living genera cannot have been acquired from the form which existed in *Phenacolestes*. Of these four, *Megapodagrion* is most like *Phenacolestes*. But *Megapodagrion* and the other three genera may equally well have descended from *Melanagrion*, with which they agree more closely in the shape of the pterostigma, although *Melanagrion* is figured as having no anal cross-veins, a not very weighty objection in this connection.

Phenacolestes might be considered ancestral to *Heteropodagrion* and *Heteragrion*, were it not that these two have, in at least some species, a greater number of postnodals.

Phenacolestes was apparently not the ancestor of:

Neuragrion, which has more postnodals, and the cubito-anal cross-vein in a different position, *i.e.*, proximal to the level of the first antenodal;

Mesagrion, for the second reason just given for *Neuragrion*;

Thaumatoneura, *Paraphlebia*, or *Dimeragrion*, which have more than one cubito-anal cross-vein and denser venation in the wing generally (*Thaumatoneura*), or between Cu_1 and Cu_2 (*Paraphlebia*), or as shown in the greater numbers of postnodals (all three);

Philogenia, which has more postnodals and rather denser venation between M_{1+2} and M_3 ;

Rhipidolestes, which has M_3 separating from M_{1+2} more proximad, R_1 beginning proximad to subnodus, and more postnodals;

Podopteryx, which has a denser venation between M_3 and M_4 and posterior to Cu_2 , and more postnodals;

Argiolestes, which has a denser venation between M_3 and M_4 .

Comparisons with genera of the Old World having more reduced venations are useless, as the equally possible descent from other extinct genera than *Phenacolestes* arises again.

Whether *Megapodagrion* and its near ally *Allopodagrion* are to be looked on as descendants of *Phenacolestes* or of *Melanagrion*,

it is of interest to point out that they are not in their present geographical distribution the nearest Podagrionine genera to Colorado, as none of their species are known from a more northern locality than Colombia ("Bogota"). In the intervening territory are found *Thaumatooneura*, *Paraphlebia*, *Philogenia*, *Heteragrion*, and *Perilestes*, and of these four genera the most northern known representatives are *Paraphlebia zoe* and *hyalina* and *Heteragrion chrysops* of the State of Vera Cruz, Mexico.⁹⁸

Professor Cockerell has suggested in correspondence that although the genera allied to *Phenacolestes* are now Neotropical, they are of northern origin or had northern ancestors. Considering how widespread are the living genera of the legion Podagrion, it seems inconceivable that their forerunners in the Miocene did not inhabit many other regions than Colorado. The fact that we know them only from Florissant seems to the writer very negative and insufficient evidence on which to found a conjecture as to the place of origin of this group.

ON THE SUBFAMILY DYSAGRIONINÆ COCKERELL.

Preceding his original description of *Phenacolestes mirandus*, Professor Cockerell proposes a new subfamily of Agrionidæ (*sensu strictiori*), under the name Dysagrioninæ, which he defines as follows.⁹⁹

"Antenodals 2.....Subfamily Agrioninæ.
Antenodals 4 or more.....Subfamily Dysagrioninæ."

The number of antennodals is hardly a character of sufficient importance by itself to establish a subfamily, owing to its variability, and at least three genera come between Professor Cockerell's two divisions. Thus in *Thaumatooneura* M'Lachn., the number of antennodals varies from 3 to 5, and *Neurolestes* Selys of Africa and *Neuragrion* Karsch of Ecuador have three antennodals.¹⁰⁰ It is not without bearing on the present topic that all three of these living genera, as well as *Phenacolestes*, are of Selys' legion Podagrion, but if any new taxonomic arrangement, whether of "subfamilies" or of "legions," is to be made, the distinctive characters must be formulated in some other terms than those which Professor Cockerell has employed. The tenability of the subfamily Dysagrioninæ has also been discussed

⁹⁸ Calvert, *Biol. Centr. Amer. Neur.*, pp. 60, 61, 64, 1901.

⁹⁹ *Bull. Amer. Mus. Nat. Hist.*, XXIV, p. 60.

¹⁰⁰ Cf. Calvert, *Ent. Mo. Mag.*, XXXVIII [2-XIII], pp. 29-32, 1902, where also the characters of the Agrioninæ are discussed.

by Mr. Champion in his preceding paper on "The Antenodal Reticulation of the wings of Agrionine Dragonflies."¹⁰¹

ADDITIONAL DATA ON DIMERAGRION N. GEN.

In addition to the venational characters which have been given on pp. 238-239, *anted*, the following are generic features from other parts of the body:

Second antennal joint one and one-half times as long as the first, the third longer than the first two together.

Ligula (median labial lobe) bilobed in its distal third, apices tapering but not very acute, separated by an interval whose width is about equal to its depth.

Tibial spines longer (often 2-3 times longer) than the intervals separating them, 7-8 in the anterior (outer) row, and 8-9 in the posterior (inner) row of the third tibia.

The generic name proposed is in allusion to the fact that there are two supplementary sectors only in two areas of the wing, viz., those between M_{1+2} and M_2 and between R_1 and M_3 .

Type, Dimeragrion percubitale n. sp.

Dimeragrion percubitale n. sp. Pl. XIV, figs. 6-8.

Adult ♂.—Vertex, genæ, and rear of the head black with some metallic green reflection not very brilliant. A yellowish streak between each antenna and the lateral ocellus of the same side. Traces of pruinosity on the rear of the head. Frons, nasus, and upper half of rhinarium dark metallic violet, well-defined from the black above and the pale yellow below, which latter forms a transverse band on the lower half of the rhinarium and basal half of the labrum, this band having almost parallel edges. Distal half of the labrum and the mandibles dark metallic violet. Submentum, mentum, and bases of the maxillæ luteous, the distal parts of these two pairs of appendages blackish. Antennæ black.

Prothorax black with a slight metallic green reflection.

Mesepisterna and mesepimera metallic green, lower part (more than half) of the former and the third fourth (counting from above) of the latter pruinose, or the latter without pruinosity. Metepisternum metallic green or metallic violet, a narrow pale (luteous?) stripe separating this color from that of the mesepimeron, or this pale stripe absent (still older males). Metepimeron and metasternum pale brown or luteous, some pruinosity on the latter.

¹⁰¹ These PROCEEDINGS, *anted*, pp. 220-224.

Legs blackish, some pruinosity on coxæ, trochanters, and femora.

Wings uncolored, the venation described on p. 238. Stigma dark brown, surmounting 2+ to 4 cells, its proximal side very oblique, so that one or more postnodals are placed between it and the costa, and at least twice as long as the distal side. The relative lengths of the four sides of the stigma, beginning with the longest, are: posterior, proximal, anterior, distal. No brace-vein, *i.e.*, no thickened cross-vein in prolongation caudad of the proximal side.

Dorsum of abdominal segments 1 and 2 dark metallic green, of 3-10 black, except that in the middle of 3-5 or 6 the black is replaced by ill-defined dark brown. Sides of 1 and 2, a transverse basal ring on 3, sides inferiorly of 3-7, pale brown or luteous. The dorsal black of 3-6 is continued ventrad at the posterior ends of these segments, forming a transverse apical band on each. A transverse basal yellow ring on 4-7. Intersegmental articulations of 7-10 luteous, otherwise 8-10 are wholly black, 9 being also pruinose on the dorsum. Pruinose traces are also visible on the intersegmental articulation of 1-2 and on the sides and sternites of most of the segments. Hind margin of 10 entire.

Superior appendages longer than 10, a little shorter than 9, black, partly pruinose. Viewed in profile, the axis of each appendage is parallel to that of the abdomen, but the superior margin is strongly declined in the proximal fourth, less so in the remaining three-fourths; the inferior margin in the distal three-fourths of the appendage is produced ventrad into a strongly convex lamella which terminates at the blunt apex of the appendage; this lamella reaches its maximum at one-half to two-thirds of the appendage-length. On the dorsal surface of the appendage at the extreme base is a short transverse ridge, which in profile view appears as a tooth. In dorsal view, each superior appendage shows its superior margin as almost straight and decreasing in width throughout the proximal three-fourths, while the distal fourth is strongly curved toward its fellow of the opposite side; the ventral lamella is inclined inward and downward (mesad and ventrad), the short transverse basal ridge is at the mesal margin.

Inferior appendages about seven-eighths as long as the superiors, black. Each one, in profile view, conical, tapering more rapidly on the superior margin beyond two-fifths its length, so that at this point this margin shows an angle; distal third curved strongly upward (dorsad) and terminating in a slender very acute apex. In ventral view, each appendage is applied against its fellow for the proximal three-fifths of its length, beyond which it is continued along the outer

(ectal) edge as a blunt cylindrical process one-half to one-third as wide as the proximal part of the appendage; the abrupt contraction from the proximal part to the distal process is made by a right-angled "shoulder."

I have not been able to detect any differences either in these appendages or in the genitalia of the second abdominal segment of the males from the four different localities.

Young ♂.—Differs from the adult ♂ in the absence of pruinosity, in the paler hue of the yellow areas generally, in having pale yellow where the adult has luteous; metallic violet on the head paler, bases of the mandibles and a spot between each one and the adjacent eye yellow, metallic violet or metallic brown instead of metallic green on the thorax, an indistinct narrow yellowish stripe on each side of the mid-dorsal thoracic carina, a bright yellow line on the humeral suture, coxæ and trochanters pale yellow, pterostigma paler brown with a pale luteous line just within each bounding vein, dorsum of abdominal segments 1 and 2 metallic blue, of 3-7 dark brown, darker at the hind end of each where it is continued ventrad as in the adult, most of the ventral surfaces of 7-10 pale yellow, except at the hind ends of 7 and 8 which are blackish, proximal half of the inferior appendages yellowish ventrally.

♀. Differs from the adult ♂ as follows: Dark color on the nasus, rhinarium and labrum shining black instead of metallic violet. Sides of the first two (confluent with the transverse yellow band below), bases of the mandibles and an oblique band running from each eye upward (dorsad) and inward (mesad) on to the anterior surface of the frons to a variable distance (not correlated with amount of pruinosity on body), bright yellow. A narrow stripe on the humeral suture, a wider stripe on the first lateral suture and on the metepisternum bordering the second suture, yellowish. Pruinosity on the thorax in slight quantity on the sterna and metapleuron, or on the sterna only. Coxæ and trochanters yellow. Pterostigma as described for the young male, pruinose on the under surface. No pruinosity (or but a trace of it) on the dorsal side of abdominal segment 9, but present in slight traces on the sides inferiorly of 1-8 and in somewhat greater amount on the sternites of 1-8. Hind margin of 10 with a narrow mid-dorsal notch about one-fourth as deep as the length of the segment, from which runs a low median carina to the anterior end of the segment; ventral surface of this segment pale brown. Abdominal appendages about twice as long as segment 10, not as long as segment 9, straight, slender, tapering to an acute apex, black.

Genital valves (=external gonapophyses of 9) reaching almost, but not quite, to the level of the hind end of 10, brownish, ventral margin spinulose, the palps slender, reaching beyond the level of the hind end of 10, but not of that of the anal tubercle (=11), darker in color.

Of the two females from Tumatumari, one lacks all pruinosity on the body and the pterostigmata, the other has a small quantity on the rear of the head only; they appear to be the most immature of their sex in the present material.

Dimensions.—Abdomen ♂ 26.5–34, ♀ 24.5–25.5; hind wing ♂ 21.5–25, ♀ 20–22.5 mm.

Habitat.—British Guiana: Wismar, January 30 and 31, three males, three females; Rockstone, February 1, six males, one female; Tumatumari, February 5, one male, February 7, one female, February 11, one male, one female; Potoro Landing, February 10, three males. Collectors, L. A. and E. B. Williamson and B. J. Rainey, 1912.

Type.—A male from Wismar, January 30, abdomen 30.5, hind wing 22.5 mm. long, in the collection of Mr. E. B. Williamson, of Bluffton, Ind. The other material in the collections of Mr. Williamson and of the author.

Habits.—The Tumatumari female of February 11, the youngest of her sex of the present material bears this label by Mr. E. B. Williamson: "taken at top of trail 2½ mile past Tiger Creek trail on twig." A male from Potoro Landing, the smallest of his sex in all the present lot has this note by the same collector: "On ends of twigs wings expanded usually in shade."

Mr. Williamson has kindly furnished the following notes with reference to the habits: "At Wismar back from the river, across the railroad tracks and south of the eminence on which stands the manager's (Mr. Croombie's) house, is a brushy swampy woods, traversed by numerous small streams which head in small ravines with abrupt and rocky ends or pockets. These streams at the season I was there were small and frequently disappeared and hence were followed with some difficulty in their upper course. All were short and were intermingled in the lower (east) end of the woods where the ground was swampy and the footing treacherous. The Agrionines were found usually near the upper ends of the streams below the rocky pockets.

"At Rockstone the railroad track was followed back toward Wismar where two or three streams, small at this season, cross the track at intervals, flowing in a northerly direction. These streams were

followed in the direction of their flow till each was lost in the woods and it was impossible to trace its course further.

"The habits of the Agrionines at both locations (Wismar and Rockstone) were identical. They rested on the extreme tips of dead twigs, usually two or three feet from the ground, with outspread wings and rigidly horizontal bodies. The color and the position assumed gave the impression of alertness and self-reliance. The flight was swift, and because of light-conditions also, was difficult to follow. At rest they were fairly conspicuous. When one flew at my approach I stood still and began examining the possible destinations in the direction of its flight, and thus located it."

Variations.—The smallest males are from Potoro Landing, followed in order by those from Tumatumari, Wismar and Rockstone. The order of the females by size from the smallest is not the same, owing perhaps to there being but a single specimen of this sex from Rockstone, viz.: Tumatumari, Rockstone, Wismar.

The most remarkable variation in this species is in the number of cubito-anal cross-veins, which varies from 1 to 4 on the front wings and from 1 to 5 on the hind. Of the twenty specimens studied only three (all males) have the same number on all four wings, and of these three two have 2 cubito-anal cross-veins and the third three. Two other specimens (females) agree with each other in the number and arrangement of these veins on their four wings, leaving fifteen out of the twenty no two of which are exactly alike in this feature when all four wings are considered. The remarkable nature of this variation will be appreciated when it is recalled that in the great majority of the Agrioninae of de Selys there is but a single cubito-anal cross-vein on each wing. The conditions found in the present material are summarized in the following

TABLE II.

Showing the Number of Wings of Twenty Individuals of Dimeragrion percutibile having the Specified Numbers of Cubito-anal Cross-veins.

(The numbers in parentheses are those of the wings in which the most proximal of these cross-veins is situated proximad to the level of the first antenodal.)

Right front wing.	Left front wing.	Right hind wing.	Left hind wing.
1 with 1 cu-a c-v	3 with 1 cu-a c-v	2 with 1 cu-a c-v	0 with 1 cu-a c-v
13 (1) " 2 " "	11 (1) " 2 " "	10 " 2 " "	8 " 2 " "
5 (1) " 3 " "	5 (4) " 3 " "	6 " 3 " "	8 (3) " 3 " "
1 " 4 " "	1 (1) " 4 " "	2 (1) " 4 " "	3 (2) " 4 " "
			1 (1) " 5 " "
20 (2)	20 (6)	20 (1)	20 (6)

It is rather curious, although at present insignificant, that the abnormality of having a cubito-anal cross-vein situated *proximal* to the level of the first antenodal should be more frequent on the left side in both the front and the hind wings.

The specific name proposed, *percubitale*, is in allusion to the unusual numbers of cubito-anal cross-veins.

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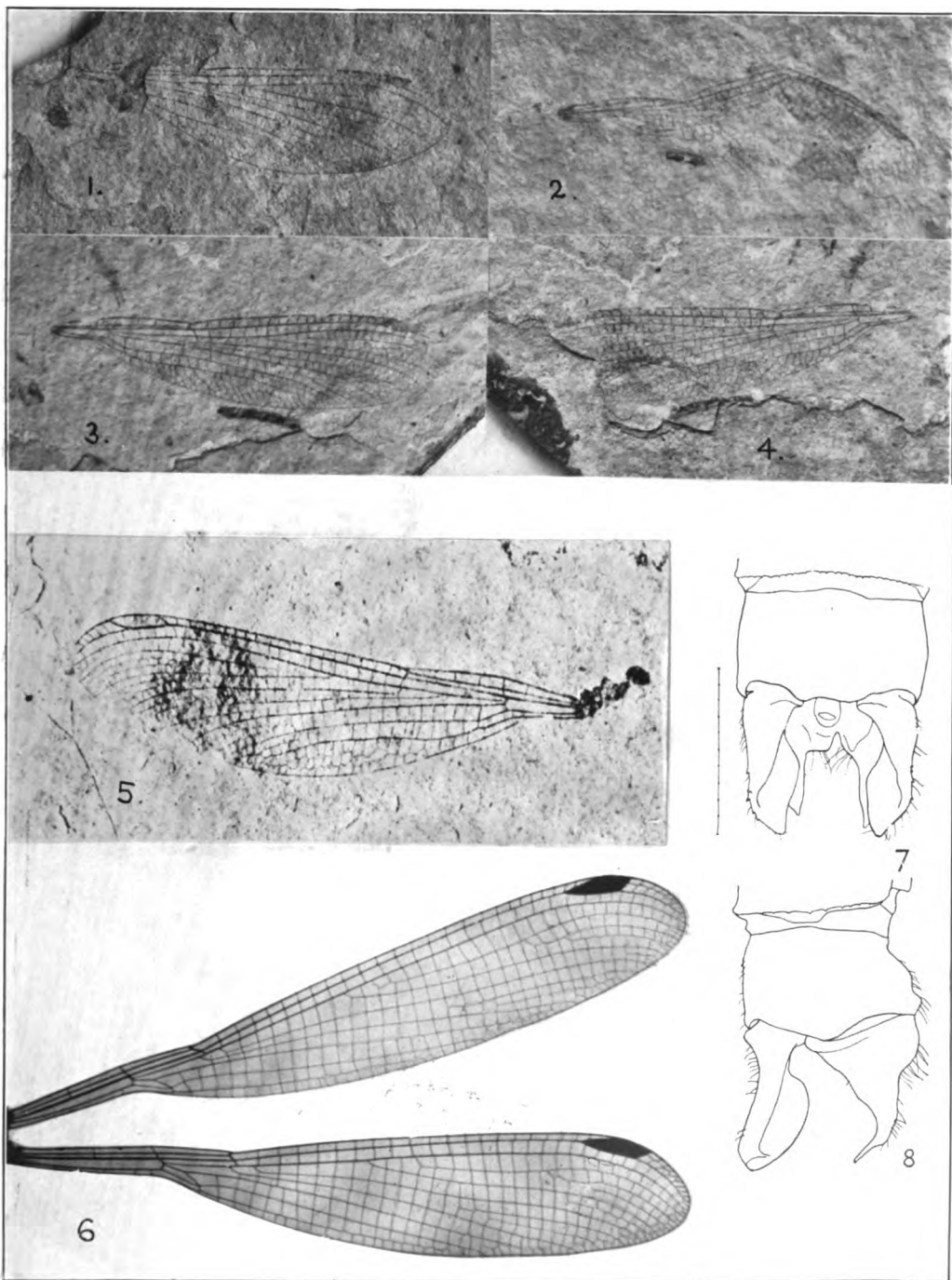
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EXPLANATION OF PLATE XIV.

- Fig. 1-5.—*Phenacolestes mirandus* Cockerell. Florissant, Colorado. From photographs. These are the specimens described *anted*, pp. 225-230.
- Fig. 6.—*Dimeragrion percubitale* n. gen., n. sp. Wings of male, Rockstone, British Guiana, February 1, 1912. (From photograph by H. A. Walters.)
- Figs. 7, 8.—*D. percubitale*, apex of abdomen of male type, Wismar, British Guiana, January 30, 1912. 7, dorsal view; 8, right side profile view. Camera drawings, Zeiss oc. 2, objective A with lower lens off. Scale alongside fig. 7 is that of one millimeter in tenths, for figures 7 and 8 only.



The Species of *Nehalennia* (Odonata),

Including one from the eastern United States hitherto undescribed.

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The genus *Nehalennia* was established by de Selys in 1850 for the European *Agrion speciosum* Charp. In his synopsis of the legion Agrion in 1876 he included within *Nehalennia* the following seven species: *atrinuchalis* Selys (Shanghai), *speciosa* Charp. (Europe), *irene* Hagen (United States), *sophia* Selys, 1876 (Province of Minas, Brazil), *posita* Hagen (United States), *denticollis* Burm. (Mexico), and *lais* Selys (Mexico). In 1895 Morse described *N. gracilis* from Massachusetts. Calvert removed *denticollis* to *Ischnura* Charp. in 1898 and *lais* to *Anisagrion* Selys in 1902. In 1903 Needham transferred *posita* to *Ischnura*. Five described species thus remain in *Nehalennia*. References to the literature are given in the catalogues of Kirby and of Muttkowski. Dr. E. M. Walker has just published the differentials of the larvae of *N. irene* and *gracilis*.*

*Can. Ent., XLV, p. 61, pl. I, figs. 1-3. June, 1913.

Some years previous to 1908 I had ascertained the presence of another species of this genus in the United States and had noted the principal characters distinguishing it from *irene*, *gracilis* and *speciosa*. On this basis, I included in my notes, furnished to the late Prof. John B. Smith in 1909, for the List of New Jersey Insects then in preparation, the record of one female from Malaga, N. J., June 27, 1908, by Mr. V. A. E. Daecke as *Nehalennia integricollis* Calv.* My absence for a year in Costa Rica speedily followed and the publication of the description of *integricollis* was forgotten, so that the name has remained a *nomen nudum*. A recent inquiry by Mr. W. T. Davis as to the place of description has recalled the matter to my mind, hence the present paper.

The species of *Nehalennia* are summarized in the following synopsis: two of them, *atrinuchalis* Selys and *selysii* Kirby (Cat. Odon., 1890, p. 147, for *sophia* Selys 1876; nec *sophia* Selys 1840 = *speciosa* Charp., 1840), are known to me by descriptions only.

- I. Hind margin of prothorax convex, entire or nearly so ♂ ♀. Superior appendages ♂ longer than inferiors.

Thoracic dorsum dull metallic green reaching laterad to beyond the humeral suture, with reddish humeral and antehumeral lines. Three antenodal cells. ♂ Abd. segs. 8 and 9 blue, 10 black dorsally, its hind margin broadly and shallowly concave; superior appendages subconical, a little recurved toward each other as if compressed. ♀ Abd. segs. 8 and 9 bronze dorsally. Abd. male 20-22, female 22; hind wing, male 13, female 16 mm. (From de Selys' description.)...*atrinuchalis*

Thoracic dorsum metallic green to beyond the humeral suture which often has a short pale stripe on its upper and another on its lower end. Three antenodal cells. ♂ Abd. seg. 8 dark metallic blue or metallic green with a pale blue mid-dorsal spot on the posterior third to half and the sides inferiorly pale blue; 9 chiefly pale blue, a broad dark metallic green or metallic blue band each side in proximal two-thirds; 10 pale blue, its hind dorsal margin cleft in the middle, a short process directed upward and caudad on each side of the cleft, each process spinulose at tip, margin otherwise not spinulose; superior appendages increasing in thickness to the apex when seen

* Ann. Rep. N. J. State Museum 1909, p. 75. Trenton, N. J. 1910.

in profile view. ♀ Abd. segs. 8-10 colored similarly to those of the ♂ but the blue on the dorsum of 8 often reaching farther toward the base and the lateral band on 9 farther posteriorly. Abd. male 20-23, female 19-22; hind wing, male 13-14, female 14-15 mm. *speciosa*

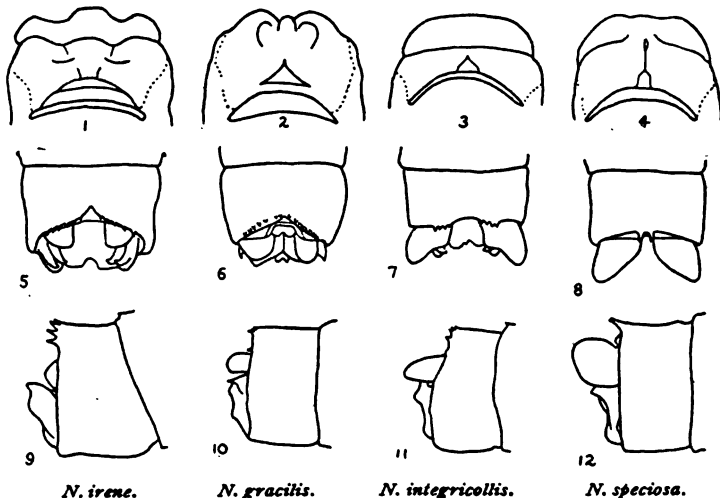
Thoracic dorsum bronze black not reaching as far laterad as the humeral suture which bears a black line. Three antenodal cells. ♂ Abd. segs. 8-10 pale blue with a bronze bilobed dorsal band on 8 and a small median spot on 9; hind margin of 10 forming two slightly marked festoons each bearing 5-6 blackish teeth; superior appendages conical, furnished at the base with an internal branch. Abd. 24-25, hind wing 15-15.5 mm. ♀ unknown. (From de Selys' description.) .. *selysii*

Thoracic dorsum ♂ bronze black reaching as far laterad as the humeral suture only at its upper sixth, the suture itself black, bordered mesad by a pale stripe one-fourth as wide as the mesepisternum on which it lies and reaching upward to five sixths of the length of the suture; of ♀ metallic green reaching laterad beyond the humeral suture which has a fine short yellow line on its upper and one on its lower end. Three (♂) or two (♀) antenodal cells. ♂ Abd. segs. 8 and 9 dark metallic blue, each with a narrow transverse pale line at base, interrupted at the middle on 8, and a narrower transverse pale line at the hind margin, sides pale blue which does not show in a dorsal view of 8 but is visible in a dorsal view of 9 especially near the hind end of the segment, no longitudinal lateral dark bands on 9; 10 pale blue with a little black at the base mid-dorsally, its hind dorsal margin concave in the middle, spinulose on each side of the emargination; superior appendages decreasing in thickness to the apex, seen in profile view. ♀ Abd. segs. 8 and 9 dark metallic blue-green, no pale dorsal spots on 8, a pale blue mid-dorsal posterior spot on 9 not half as long as the segment, sides inferiorly and 10 pale blue, the last with some traces of black or metallic green at the extreme base. Abd. ♂ 21, ♀ 18.5-20; hind wing, ♂ 13, ♀ 13-13.5 mm. *integricollis* n. sp.

- II. Hind margin of prothorax emarginated ♀, entire and convex ♂. Superior appendages ♂ equal to, or shorter than, the inferiors. Hind dorsal margin of abd. seg. 10 of ♂ cleft in the middle, spinulose on each side of the cleft. Thoracic dorsum metallic green, reaching laterad to beyond the humeral suture which is often marked as above stated for *speciosa*. Three antenodal cells.

Hind margin of prothorax of ♀ bilobed, but in front of the cleft separating the two lobes is a small more nearly erect process

which partly fills the cleft when the prothorax is viewed from in front, a median pair of slender approximated mesothoracic processes just behind the prothorax. ♂ Abd. seg. 8 blue with a transverse dorsal basal black or metallic green line or narrow stripe, 9 and 10 entirely pale blue; superior appendages nearly as long but not as high as the inf. apps., seen in profile view. ♀ Abd. seg. 8 pale blue with a transverse basal stripe and a lateral band reaching caudad therefrom dark metallic green or black, or, the dorsum of 8 almost entirely blue-black, sides inferiorly pale (1♀), 9 blue with a triangular black band each side in proximal half of segment, 10 entirely blue. Abd. ♂ ♀ 20-22; hind wing ♂ 13-14.5, ♀ 14-15 mm. *gracilis*



Figs. 1-4, hind part of prothorax of females viewed from in front and a little above, the head of each specimen having been removed to see the prothorax clearly; the dotted lines show the boundaries of the median metallic green and the lateral pale green or yellow. Figs. 5-8, dorsal views of abdominal segment 10 and appendages of males. Figs. 9-12, profile views, right side of the same. All the figures drawn to the same scale with the camera lucida, specimens illuminated by direct sunlight.

N. irene, ♀, Lehigh Gap, Pennsylvania, July 13, 1900; ♂, Berlin, New Jersey, July 17, 1893. *N. gracilis*, ♀, Lehigh Gap, Pa., June 29, 1897; ♂, Sherborn, Massachusetts, June 24, 1895 (co-type of Morse). *N. integricollis* n. sp., ♀, Enterprise, Fla., April 18, 1896 (type); ♂, Haulover, Fla., March. *N. speciosa*, ♀ ♂, Robenhausen, Switzerland, July 3, 1904.

Hind margin of prothorax of ♀ trilobed, no slender mesothoracic processes as described for *gracilis* but the mesostigmal laminae are larger and more projecting than in that species. ♂ Abd. seg. 8 dark metallic green, a mid-dorsal spot on the posterior fourth or less and the sides inferiorly pale blue, 9 pale blue with a triangular dark metallic green spot on each

side of dorsum in its proximal half or more, 10 pale blue with a metallic green spot each side of dorsum at base; superior appendages much smaller than the inferiors, each of the latter with two black-tipped hooks. ♀ Abd. segs. 8 and 9 dark metallic green, sides inferiorly pale, 9 with a mid-dorsal anteapical pale blue spot, *or*, more rarely, 8 and 9 colored as in the male, 10 as in the male. Abd. male 20-23, female 20-22.5; hind wing male 13-15.5, female 14-15 mm. irene

Of *Nehalennia integricollis* I have seen five females from Florida, one from Thomasville, Georgia, April 28, 1903, by Mr. Morgan Hebard, and the one from Malaga, New Jersey, mentioned above. Of the five Florida females, one, the type of the species, is from Enterprise, April 18, 1896, by Mr. Philip Laurent; it is the specimen cited in Ent. News, vii, p. 302, as *N. irene* Hag.; two others, exact locality not recorded, are due to Dr. D. M. Castle in 1903 (cf. Ent. News, xiv, p. 244); a fourth, "Fla. exchange," has long been unidentified in the collection of the Academy of Natural Sciences of Philadelphia, while the fifth, from Enterprise, May 17, belongs to the Museum of Comparative Zoology, Cambridge, Mass. Excepting this last, these females are in the collection of the Academy named.

The single male referred to this species, from Haulover, Florida, in March (Museum of Comp. Zool.), may not be conspecific with these females, as the extent of dark coloration on the thoracic dorsum and the number of antenodal cells are different, as has been indicated above. It resembles the description of *N. selysii* Kirby (*sophia* Selys 1876) in many respects, but is smaller, has the proximal and distal sides of the pterostigma nearly parallel (proximal side more oblique than the distal in *selysii*), the posterior or inferior side of the quadrilateral of front wing more than twice as long as the anterior or superior side ("l'inferieur moitié plus long que le supérieur" in *selysii*), labrum with a transverse basal black band ("un point médian enfoncé" in *selysii*), nasus entirely black. The abdomen is not fully colored, 3-6 being of a pale brown with a narrow transverse basal pale blue ring; other differences in abdominal coloring may be seen from the

above synopsis. The appendages may be quite similar to those of *selysii*. The known habitats of the two forms are far apart.

It will be noticed that, in spite of its geographical distribution, *integricollis* is nearer structurally to the European and Brazilian species than to the two already known from the United States.

Dr. F. Ris, within recent years, has described* color varieties in Swiss specimens of *N. speciosa* which may possibly indicate a color dimorphism or polymorphism among the females analogous to that known in *Ischnura*. In this connection it is of interest to note that I found a single female of *N. irene* from Manchester, Maine, June 12, 1899, by Miss Mattie Wadsworth (now in the collection of Mr. E. B. Williamson†), which has abdominal segments 8 and 9 colored as in the male of *irene* and unlike the majority of females as stated above. The alternative coloring of segment 8 in the female of *gracilis* mentioned above is seen in one specimen from Iona, New Jersey, July 13, 1902 (Daecke).

The transference of species from *Nehalennia* to *Ischnura*, mentioned in the first paragraph of this paper, indicates the difficulty of sharply distinguishing these two groups. Even now it does not appear to be possible to give any one character which will invariably separate them. The following statement may be of assistance:

Nehalennia. Prevailing color of dorsum metallic green. Second joint of antenna black, its middle third pale. Pale color on postero-superior part of head forming a transverse line whose extremities are not enlarged (except in ? *integricollis* male). M₂ on the hind wings separating from M₁ more often nearest the fourth postnodal. Males without an elevated, bifid, dorsal process on abdominal segment 10 (ap-

* *Farbenvarietäten der Agrionide Nehalennia speciosa* Charp. Mitt. schweiz. entom. Gesell. XI, pp. 159-165, 1906. Very recently this species has also been discovered in Asia by Bartenev, Zool. Anz. xxxv, p. 278, 1910; Ann. Mus. Zool. Acad. Imp. Sci. St. Petersburg. xvi, p. 447, 1912.

† Mr. Williamson kindly placed his collection of *Nehalennia* at my disposal for study.

proach thereto in *speciosa*), stigmata of front and hind wings similar in size, shape and color. Females without a vulvar spine.

Ischnura. Prevailing color of dorsum not metallic green. Second joint of antenna otherwise colored. Pale color on postero-superior part of head forming two round or cuneiform postocular spots sometimes united by a transverse line. M₂ on the hind wings separating from M₁ more often nearest the third postnodal (except in *I. erratica*, *I. cervula*, *I. in-armata* and possibly others). Males with an elevated, usually bifid, dorsal process on abdominal segment 10, stigmata of front and hind wings dissimilar in color, size or both (dissimilarity slight in *I. posita*). Females with or without a vulvar spine, in some cases even in the same species.

**The True Male of *Nehalennia integricollis* and *N. pallidula*, n. sp.
(Odon.).**

In describing *Nehalennia integricollis* in the NEWS for July last, page 312, from a female type, a male from Haulover, Florida, was doubtfully referred to this species and the differences, other than sexual, between it and the type were pointed out, page 314. On June 27 and July 2, at Malaga, New Jersey, I obtained three pairs of *integricollis* and a number of other individuals of both sexes. The males so obtained are the true *integricollis*, are much like the females in color, and consequently quite different from the Haulover male. To the Haulover male a *new name* must therefore be assigned, and *PALLIDULA*, in allusion to the narrower metallic green area of the thoracic dorsum is suggested; figures 7 and 11, page 313, illustrate the appendages of *pallidula* male.

Some additional notes on *integricollis* follow. The words "Superior appendages ♂ longer than inferiors," must be stricken out of the character of group I, page 311, as in true *integricollis* ♂ the superior appendages are not longer than the inferiors, although this is the case in *atrinuchalis*, *speciosa*, *selysii* and *pallidula*.

In the recently killed *integricollis* ♀ the eyes are olive green above becoming bluish-green on the middle third and yellowish-green below. Genae below the level of the base of the antennae very pale blue and a slender finger-like prolongation of this color runs upward each side into the dark metallic green of the rear of the head. The anterior surface of the frons, the rhinarium, the bases of the mandibles and the labrum are pale bluish, the last with a transverse basal black stripe. The pale transverse occipital line not enlarged at its extremities. The sides of the thorax and of abdominal segments 1-7 or 8 greenish-yellow, or in the teneral pinkish.

In the recently killed ♂ *integricollis* the eyes are blue-black above and become increasingly paler blue to the under surface. The remainder of the head is colored as in the ♀ described above. The metallic green of the dorsum of body generally (including the head), is darker than in the ♀ owing, partly at least, to the admixture of more purple. Thoracic dorsum metallic green reaching laterad beyond the humeral suture as in the ♀. Sides of the thorax and of abdominal segments 1-6 pale bluish, of 7 and 8 yellowish, or of 8 bluish. Dorsum of abd. seg. 8 dark metallic green with no pale dorsal spots; 9 and 10 blue, 9 with a metallic green stripe on each side of dorsum reaching from base (where they are narrowly confluent with each other) one-half to two-thirds way to the hind end, 10 with a transverse basal black line.

Hind dorsal margin of 10 cleft in the middle and spinulose. Appen-



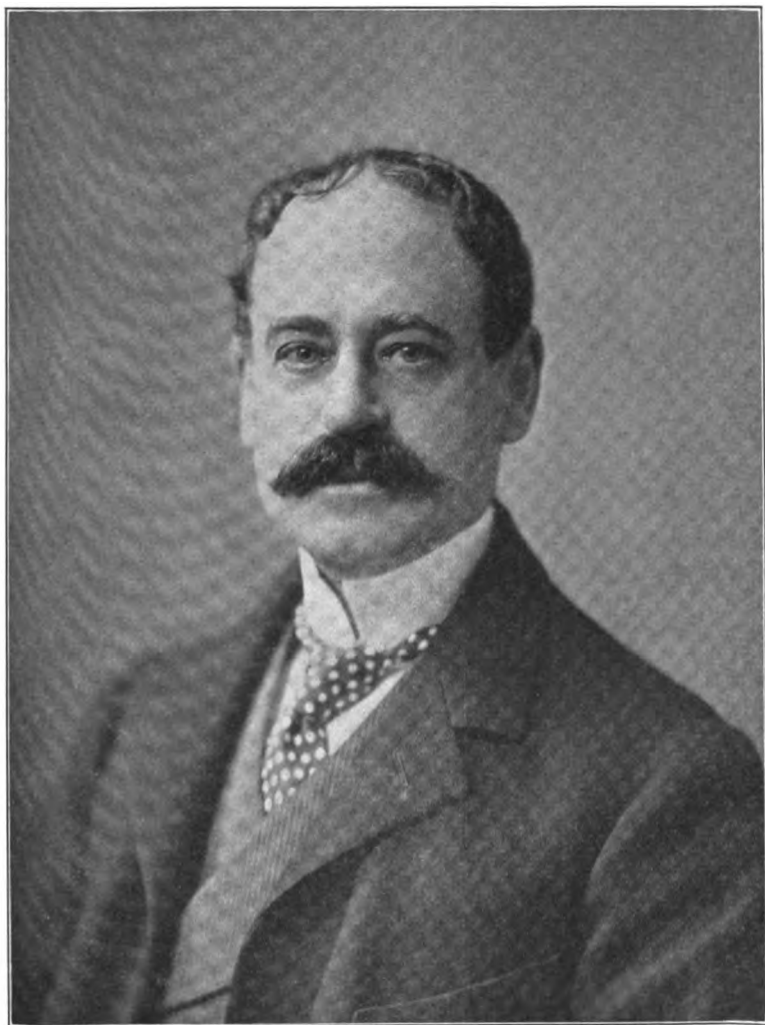
dages as shown in the accompanying figures, resembling those of *irene* and *gracilis* (cf. page 313). The male is like the ♀ also in having *two* antenodal cells. Abd. 18.5-19.5, hind wing 11.5-12.5 mm.

In the teneral ♂ the pale colors of the body including the eyes are pale violaceous.

The type of this description of *integricollis* ♂ is now in the collection of the Academy of Natural Sciences of Philadelphia.

N. integricollis was taken at Malaga, on the dates mentioned, at the southwest corner of the pond made by damming Scotland Run, a branch of the Maurice River. It was flying among the abundant pickerel weeds (*Pontederia cordata*, var. *angustifolia*) and white water lilies (*Castalia*) then in bloom and was associated with *Telagrion daeckii* and numerous *Ischnura posita*, while *Pachydiplax longipennis* and *Libellula incesta* flew above.

PHILIP P. CALVERT.



DR. HORACE JAYNE.

OBITUARY.**DR. HORACE JAYNE.**

(Portrait, Plate XII)

Dr. Horace Jayne, at one time actively interested in the study of the Coleoptera, died at Wallingford, Pennsylvania, near Philadelphia, on Tuesday, July 8, 1913, in his fifty-fifth year.

He was a son of Dr. David Jayne and was born in Philadelphia, March 17, 1859, graduated from the College of the University of Pennsylvania in 1879 and from the Medical School of the same in 1882. After studies at the universities of Leipzig, Jena and Johns Hopkins, he returned to that of Pennsylvania, where he became assistant instructor in Biology and professor of Vertebrate Morphology, 1884-1894. He took an active part in the founding of the School of Biology, serving as secretary of the Faculty thereof, 1884-1889, Dr. Joseph Leidy being director of the school. In 1889 Dr. Jayne became Dean of the College Faculty, and in 1892 of that of the Department of Philosophy also, holding both positions until 1894. From that year until 1905 he was professor of Zoology and director of the Wistar Institute of Anatomy and Biology of the same university. In later years, until 1909, he took an active editorial interest in the *Journal of Morphology*, the *Anatomical Record* and the *Journal of Experimental Zoology*.

His zoological activities for the last thirty years of his life were mainly concerned with the mammals, and his chief work was *Mammalian Anatomy, A Preparation for Human and Comparative Anatomy. Part I. The Skeleton of the Cat, its Muscular Attachments, Growth and Variations, compared with the Skeleton of Man* (Philadelphia, J. B. Lippincott Co., 1898), a stout octavo volume of 836 pages and over 500 original illustrations. This volume was intended, to quote from the preface, as "the first of a series which aims to present a more

accurate and more comprehensive description of the structure of a typical mammal than has been hitherto attempted." No other volume appeared, however.

In a brief notice which he read on April 10, 1895, at a memorial meeting for his university colleague, John A. Ryder, Dr. Jayne refers to his own work, "in arranging part of the collections of the [American] Entomological Society" at a time when he first made Ryder's acquaintance. This must have been about 1876. He became a member of the Society August 9, 1875, in the year before that body came to occupy quarters in the building of the Academy of Natural Sciences. His association here with Drs. LeConte and Horn is indicated in the opening paragraphs of his two Coleopterological articles, *Descriptions of some monstrosities observed in North American Coleoptera* (Trans. Amer. Ent. Soc. VIII, pp. 155-162, pl. IV, June, 1880) and *Revision of the Dermestidae of the United States* (Proc. Amer. Philos. Soc. XX, pp. 343-377, pls. 1-4, August 18, 1882). The latter paper is the more pretentious, and in it the author says: "The arrangement of genera is, substantially, that already well known, save only the necessary alterations incident to the introduction of two new genera, [*Acolpus* and *Axinocerus*]. The specific classification is almost entirely original." Three new species of *Attagenus*, one of *Acolpus*, two of *Trogoderma*, one of *Axinocerus*, were described. In both of these papers the author's name appears as Horace F. Jayne, but he dropped the "F." in later years.

Dr. Jayne, in virtue of his position as secretary of the Faculty of Biology of the University of Pennsylvania, in 1888, will ever be associated in my memory with the happy beginnings of my own collegiate studies in zoology and with his willing assumption of the task of major examiner for the doctor's degree, a post made suddenly vacant by the death of Ryder. Those kindly recollections have remained unclouded, and it is with genuine sorrow that this brief notice is written in some slight acknowledgment of him who has passed away.

P. P. C.

Studies on Costa Rican Odonata.

V. The Waterfall-Dwellers: *Thaumatoneura* imagos and possible male dimorphism.

By PHILIP P. CALVERT, PH.D., University of Pennsylvania, Philadelphia, Pa.

(Plate XIV.)

HISTORY.

In June, 1897, the late Robert McLachlan, of London, described a new and remarkable dragonfly under the name *Thaumatoneura inopinata*. He introduced his description with these words: "At the recent sale of the collections formed by the late Mr. Reginald Cholmondeley, of Condover Hall, Shrewsbury, I obtained the few *Odonata*, solely on account of a large Calopterygine, which was evidently something unknown, but the remarkable nature of which I did not fully realize until after it came into my possession. The former owner of the collection valued insects and other natural productions, solely for their beauty or forms, and cared little for names, and nothing for localities, so I am unable to say whence this specimen came; but as the pin (or rather skewer!) was similar to those used for some other insects in the same collection which were Chinese or Japanese in origin, I hazard a conjecture that it may belong to the same region." In August,

1900, however, he was able to state that this insect belonged to the New World, as M. Martin possessed a specimen from Chiriqui, in Panama.

About 1903 Mr. C. F. Underwood collected at Carrillo, Costa Rica, six males of *Thaumatoneura* and sent them to London, where they were acquired by Dr. F. D. Godman, and sent to me for study. Four of the six were the *inopinata* of McLachlan with the middle of each wing crossed by a broad, dark brown band. The other two had the wings uncolored, wherefore they received the name *Thaumatoneura pellucida* (Calvert, 1904).

On June 23, 1909, the first day of our first stay at Juan Viñas, Costa Rica, our lepidopterological friends, Messrs. William Schaus and J. Barnes, gave us some Odonata which they had taken there for us, including some males of both *inopinata* and *pellucida* and two female *Thaumatoneurae*, so different from the males that it was impossible to determine to which of the two species they should be referred; they had the *tips* of all the wings dark brown. Thereafter, *Thaumatoneura* became for us one of the principal attractions of Juan Viñas, that delightful spot which, as readers of the NEWS are already aware, furnished us with the remarkable larvae of *Cora*, of *Mecistogaster modestus*, and of other species still to be described.

HABITATS AND HABITS.

Of all the localities in Costa Rica which we visited, Juan Viñas was the only one where we ever saw *Thaumatoneura*. We were not at Carrillo, but in many respects the Odonate fauna of this place, judging from Mr. Underwood's collection recorded in the *Biologia Centrali-Americana*, is very similar to that of Juan Viñas. The former is on the northern, the latter on the southeastern slope of the volcano Irazú and their respective altitudes are 300 and 1040 metres (980 and 3400 feet). *Thaumatoneura* must surely live in still other places in Costa Rica.

In our experience *Thaumatoneura*, as adult or as larva, was never more than a few yards distant from a waterfall and was most abundant within its spray. The sides of the canyon

of the Rio Reventazon at Juan Viñas descend steeply from 1200 to 760 metres (4000 to 2500 feet) at the river, and down the canyon side tumble many small streams and rivulets, often in cascades and in falls of different heights. Where there is slant enough the wet rocks nearby are draped with mosses, maiden hair ferns, begonias, dwarf Selaginellas, Tradescantias, a *Streptocarpus*-like plant, etc., or in quieter more sheltered places are Melastomes (*Miconia*), *Commelina*, etc. Only at three such falls did we ever observe *Thaumatoneura*, two of them at altitudes of about 3300 feet, the third at about 2500 feet. They were most abundant on the whole at one of the former two falls, one whose perpendicular height was the greatest of the three, perhaps 200-300 feet. We were puzzled why these insects should not be found also at other streams under generally similar conditions. An explanation of their absence from one of these streams, the little Rio Naranjo, is suggested in our diary: "At the Naranjo I saw a number of lizards from two inches to a foot in length and these possibly keep down the number of insects. At our two waterfalls [*i. e.* where we frequently found *Thaumatoneura*] we have seen no lizards."

We observed the imagos of *Thaumatoneura* in June (23-28), July 21 to August 3, September 28 and 29, when there was an evident decrease in their numbers; October 1 and December 2, 1909. In February (14-18), and March (21), 1910, none could be found, but they were again visible in April. In June, 1909, the males of *pellucida* appeared to be less numerous than those of *inopinata*. All the males of *Thaumatoneura* observed in July were pruinose, but two teneral females were taken on August 1.

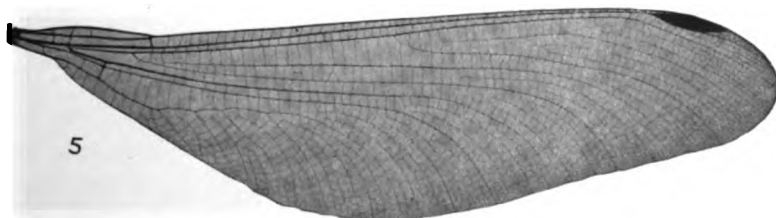
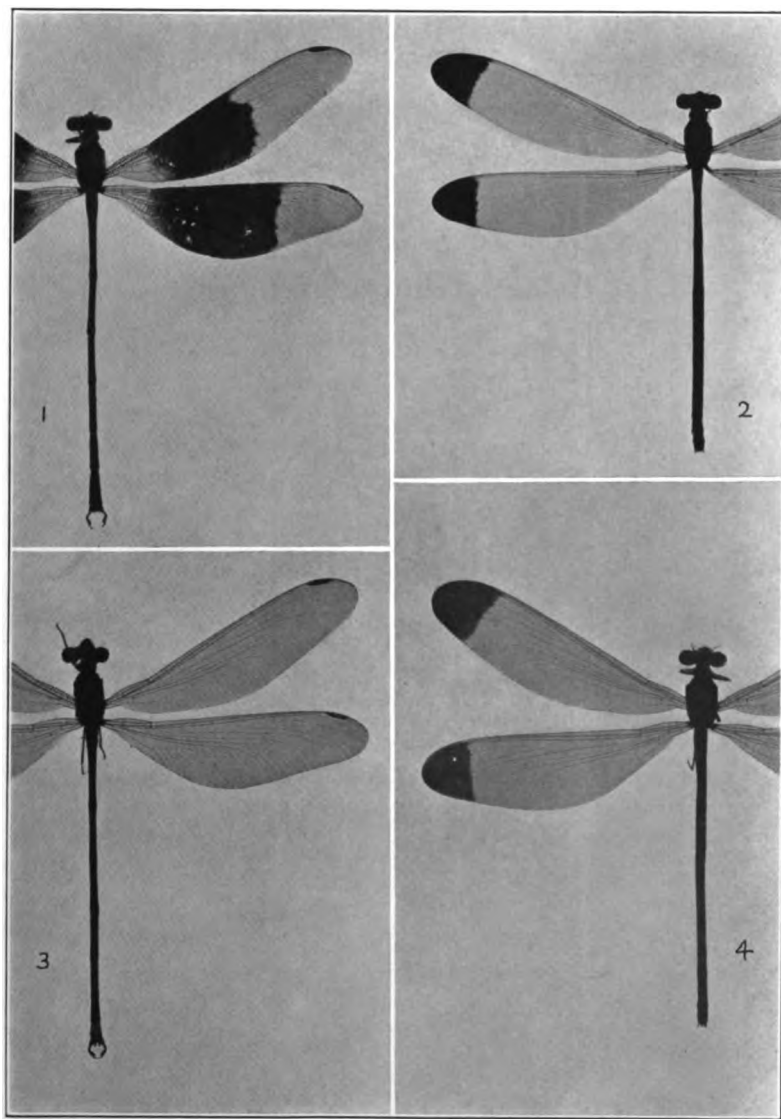
"Males, at least, often remain on the same spot for a long time without moving—I should say for half an hour or longer. Females seem less persistent, but one I timed was ten minutes on the same bit of twig. Except in pairing males, the wings of both sexes, when at rest, are held in the vertical position, folded together but every now and then an individual will open and then close its wings once or several times in succession." "Standing at the bottom of the waterfall and looking up

through the spray and over the vegetation which carpets the almost perpendicular rocks, I could see the *Thaumatoneuras*—two species (*inopinata* and *pellucida*)—fluttering through the spray, chasing each other or alighting upon the rocks and plants. So heedless of the spray are they that it gathers in little drops on the wings or hangs suspended from the hind end of the body." (June 24, 1909).

"At the farther waterfall was one male each of *T. inopinata* and *T. pellucida*. I stayed here fully an hour during which there were periods of bright sunshine, but no more *Thaumatoneuras* came, and the two mentioned remained for long periods almost motionless on twigs until I caught them." (December 2, 1909).

Owing to the habit of *Thaumatoneura* of spending most of the time over vertical wet rocks, the capture of them was often only possible by climbing up on the rocks themselves, or by hanging with one hand to some long, dangling, nearby root or vine and with the other sweeping the net.

Pairing of *T. inopinata* was observed twice on June 24 and once on June 27; of *T. pellucida* once on July 30 and again on October 1. Notes made at the time on the behavior of *inopinata* are to the following effect: The male seeks and flies after the female and seizes her by her prothorax, his superior appendages being deeply inserted behind her prothorax and between it and the mesothorax, while his inferior appendages are applied against the dorsal surface of her prothorax (this from a pair which I caught and held in my fingers). After seizing the female, the male probably applies the ventral surface of his ninth abdominal segment to the ventral surface of his second abdominal segment to charge the vesicle of the latter with sperm; of this I am not entirely sure as I think I saw it but once and the operation itself occupies very little time. Male and female then assume the usual position of the Odonata in pairing. Different pairs of *inopinata* require different lengths of time to effect the attachment of the female to the male, one pair having made four or five attempts before this was accomplished, another pair effecting it at once. In one case on June 24, 1909, where I did not see the actual begin-



1 ♂, 2 ♀, THAUMATONEURA INOPINATA,
3, 5 ♂, 4 ♀, TH. PELLUCIDA.—CALVERT.

ning of mating, it was 3 P. M.; in the other case it began at 3.45 P. M. In both cases the male held his wings horizontal and expanded, the female had hers vertical and folded together. At the end of ten minutes the male released his hold of the female and at the same instant folded his wings together into the vertical position. The female, losing her attachment by her abdomen to the male, assumed in both cases a position about half an inch below the male resting on the same twig or rock on which he was. So the two remained for about four minutes. In the first case male and female then flew away at about the same instant, but not together, she to begin egg-laying. In the second case the female flew six inches or so away and began egg-laying while the male remained for at least ten minutes on the same spot (projecting rock) on which the pairing had occurred. In the case of June 27th, after pairing the male flew away leaving the female resting on the leaf which both had occupied.

No *pellucida* were observed pairing in June and the males of this species were less numerous than those of *inopinata*. On July 30, 1909, after some hours' watching, I saw a male *pellucida* and a female fly to a dead branch on the bank or side of the railroad cutting just west of the farther waterfall and about twenty feet above the tracks. There was no doubt that they were pairing, so it was highly desirable to capture them both. The steep bank was composed of reddish soil, loose stones and soft rock, and was wet from the rains. I climbed up as far as the steepness permitted, but the distance was still too great for the length of my net stick. After two attempts to lengthen it with branches, which chanced to be lying there, but which broke under the weight of the net, the umbrella was utilized for this purpose. Again I climbed the bank, but the soft earth gave way and collector, net and umbrella slid swiftly and separately to the railroad tracks. A second attempt had the same result. A third, made more cautiously, was so successful that the net was thrown over the accommodating pair which had lingered for at least ten minutes on the same branch and by gently dragging the net down over the bank I at last got the insects safe into my hands. Then it was that I realized for the

first time that this female was so like the females which we had seen and taken pairing with *inopinata* males that a minute comparison would be necessary to determine whether there was any difference between them or not. We saw no other pairs of *pellucida* until October 1, 1909, when one was seen and obtained at the same waterfall.

What we presumed to be egg-laying was watched in three or four instances on June 24. The female made the usual abdominal movements seen in the *Zygoptera* generally, that is those adapted for inserting the eggs into plant substances. Some of these movements were, however, over hard rock and it seems unlikely that any eggs were placed on it. At other times the movements were made against moss and roots. At times the female had her abdomen buried to half its length in the mass of wet moss. Pieces of roots and of moss in which the oviposition had apparently been made were carried to our room at Cartago and kept in water for months but we never obtained any larvae from them. The ovipositing female is not accompanied or followed by the male. Indeed once, on June 24, a female flew close to a resting male without attracting any response from him.

THE FEMALE.

We have three females of *Thaumatoneura* taken while pairing; one is that taken with *inopinata* male on June 27, 1909, the other two taken with *pellucida* males on July 30 and October 1 respectively. Nine other females were taken singly. All twelve females are compared in the accompanying table with respect to all the differences which I have been able to detect between the female of June 27 (No. 9) on the one hand, and those of July 30 (No. 11) and of October 1 (No. 12) on the other. It will be seen that these differences are very slight and that the positions of Nos. 9, 11 and 12 in the whole series are such as to render it unlikely that females of *inopinata* can be distinguished from those of *pellucida* by any of these features.

MALE DIMORPHISM.

This apparent lack of external characters separating the females pairing with *pellucida* males from that pairing with *in-*

COMPARISON OF THAUMATONEURA FEMALES ARRANGED IN ORDER ACCORDING TO THE RATIO $\frac{b}{a}$

	1 June 28	2 July 21	3 Aug. 1	4 Apr. 30	5 June 23	6 June 26	7 Aug. 1	8 May	9 June 27	10 June 28	11 July 30	12 Oct. 1
Length of left hind wing in mm. (a).....	45	46.5	49.5	49	49	49	49	50.5	46	47	49.5	48
Width of left hind wing at origin of M_2 , measured at right angles to costa, in mm. (b)...	10.5	11.5	12	12	12	12	12	12.5	11.5	12	12	12
Value of ratio $\frac{b}{a}$	.233	.247	.242	.245	.245	.245	.245	.247	.25	.265	.263	.27
Width of left hind wing at proximal edge of brown spot at tip, measured parallel to width b, in mm. (c).....	10.	10.5	10.5	10.	10.5	11.	10.5	11.5	9.5	10.5	11.	11.
Distance from tip of left hind wing to proximal edge of brown spot measured at right angles to b and c, in mm.....	9.	10.	8.5	8	9	10	9.5	10.5	8.	9.5	8.5	9
Point of beginning of two rows of cells between M_2 and R_2 with reference to the proximal edge of the brown spot at tip (front wings only)	5 cells distal	distal	3 (R) 5 (L) cells proximal	1 (R) 2 (L) cells proximal	at (R) distal (L)	at	at	at (R) 1 cell proximal (L)	at	at	4 cells proximal	3 (R) 5 (L) cells proximal
Formulae showing how the increase in density of reticulation between M_2 and Cu_1 begins (front wings only).....	2,3,8,8; 3,8,3,8; 4....(R) 2,2,8,8; 3,8,3,8; 4....(L)	2,3,8,8; 3,8,3,8; 4....(R) 2,2,8,8; 3,8,3,8; 4....(L)	2,3,8,8; 3,8,3,8; 4....(R) 2,2,8,8; 3,8,3,8; 4....(L)	2,3,8,8; 3,8,3,8; 4....(R) 2,2,8,8; 3,8,3,8; 4....(L)	2,3,8,8; 3,8,3,8; 4....(R) 2,2,8,8; 3,8,3,8; 4....(L)	2,3,8,8; 3,8,3,8; 4....(R) 2,2,8,8; 3,8,3,8; 4....(L)	2,3,8,8; 3,8,3,8; 4....(R) 2,2,8,8; 3,8,3,8; 4....(L)	2,3,8,8; 3,8,3,8; 4....(R) 2,2,8,8; 3,8,3,8; 4....(L)	2,3,8,8; 3,8,3,8; 4....(R) 2,2,8,8; 3,8,3,8; 4....(L)	2,3,8,8; 3,8,3,8; 4....(R) 2,2,8,8; 3,8,3,8; 4....(L)	2,3,8,8; 3,8,3,8; 4....(R) 2,2,8,8; 3,8,3,8; 4....(L)	2,2,8,8; 3,8,3,8; 4....(R) 2,2,8,8; 3,8,3,8; 4....(L)
Length of the abdomen (including appendages) in mm.....	50	50	51.5	54	55	54	53	53	51	53	54	53

* Taken pairing with *isopinnata* ♂. † Taken pairing with *pelticoida* males. The year of No. 4 is 1910, of all others 1909.

opinata male suggests the query as to whether we are dealing with two or one species here. A number of species of Odonata are known (not to mention other groups) in which there is one form of male and two forms of females within the same species. Such are members of the genera *Ischnura*, *Anomalagrion*, *Ceratura*, *Agriocnemis* and *Neurothemis*, *Erythrodiplax umbrata* and *funerea*. While some of these so-called dimorphic females are explicable as due to differences in age, others can not be interpreted in that way. Can *Thaumatoneura* present an inverse case in which there are two forms of males and but one form of female in the same species? This leads us to consider the differences between the *pellucida* and *inopinata* males.

When I originally described *pellucida* male, I could find only two differences between it and *inopinata* male. One of these was the absence of any coloring on the wing, the other the presence of "a transverse pale citron band on nasus and on frons connecting the yellow genal spots," this band being absent in *inopinata* male. The pale citron of dried specimens, I now know, is pale blue in life. This diversity in the coloring of frons and nasus holds true for the majority of the present examples, but of the fourteen males of *inopinata* two have the pale band very well marked and three others have indications of some pale color on these parts. All fourteen *pellucida* males have the pale band. The *inopinata* males are slightly larger, average length of the abdomen 58.7, range 55-61 mm.; hind wing, average 47.6, range 45-49 mm. (14 specimens). *Pellucida*: Abdomen, average 57.25, range 52-61; hind wing, average 46.85, range 44.5-49.5 mm. (14 specimens). I have not been successful in finding any other differences in the imagos. There is no reason to think that the two forms represent age differences because no transitional individuals (so far as the presence or absence of the coloring of the wings is concerned) have been found. We have seen both forms emerge from the larval exuviae at metamorphosis, and both forms become equally pruinose on the body although so markedly different in wing coloring.

The seasonal distribution of our males is as follows: *In-*

opinata, June 23, 6 males; June 24, 2 males; June 26, 1 male; June 27, 1 male; July 21, 1 male; July 27, 1 male; December 2, 1 male; May 2, 1 male. *Pellucida*: June 23, 1 male; June 24, 2 males; June 26, 1 male; June 27, 1 male; July 22, 1 male; July 27, 1 male; July 30, 1 male; August 3, 1 male; September 28, 2 males; October 1, 1 male; December 2, 1 male; May 1, 1 male. This list gives the actualities, not the possibilities, of capture; on many occasions the insects were not molested as we wished to observe their habits and behavior. This list does show that both forms occur at the same times and, we may add, at the same waterfalls.

Among our exuviae of *Thaumatonera* we have one from which we saw *inopinata* male emerge, another from which *pellucida* male emerged. A minute comparison of these two exuviae has failed to furnish any differences other than those due to injuries sustained by the larvae previous to transformation.

A definitive answer to the question raised on page 344 as to the relationships between the two male forms of *Thaumatonera* to each other and to the apparently single form of female, can only be obtained from breeding experiments. As far as present evidence goes, I am inclined toward the dimorphic male hypothesis. If it prove to be true, *Thaumatonera* would furnish a case more or less parallel to those described by Westwood (1839) for certain Staphylinidae, Leuthner (1885) for Lucanidae, and Skinner (1913) for *Lycaena pseudargiolus*.

ADDITIONAL DESCRIPTIVE NOTES.

Some additional notes on our Juan Viñas material of *Thaumatonera* are added.

Description of the Colors of T. pellucida male, taken Dec. 2, 1909.

Description made immediately after death. Eyes dark brown, almost black. Each gena with a round yellow spot below the base of the antenna; frons between the right and left yellow spots pale blue as also is the nasus, but the fronto-nasal suture has a black line and black encroaches on each side of the nasus from below. Thoracic sutures and mid-dorsal carina lined with orange-yellow. A short occipital line, four small spots on the front prothoracic lobe and a pair of mid-dorsal spots on the middle prothoracic lobe are also orange-yellow.

The rest of the body is black, covered with pruinosity on labium, under and lateral surfaces of thorax, most of abdominal segment 1, a

large part of the dorsum of 2, a transverse basal ring on 3-7, sides of 8, much of 9 and 10, especially on the dorsum.

Legs yellowish, articulations and tarsi blackish.

The colors immediately after death of T. inopinata male, also taken December 2, 1909, agreed with those noted above for *pellucida*, including the pale colors of frons and nasus, but there was very little pruinosity except on the ventral surface of the thorax and on the posterior abdominal segments. The dark band on the wings varies somewhat in width; its proximal edge is at the nodus, its distal edge at the costa is from 16 to 20 mm. distad on the front wings, from 21 to 25 mm. distad on the hind pair. The male of May 1, however, has the proximal edge of this band 6 mm. beyond the nodus and the distal edge at 21 and 26 mm. from the nodus on front and hind wings respectively; the costal and radial spaces between nodus and the proximal edge of the band are more or less brown.

The colors of the body of Thaumato-neura females (dry, I have no notes on living colors) are as in *inopinata* males. In none of them is a distinct pale band on frons and nasus present, such as can be seen plainly on the dried *pellucida* males; Nos. 2, 5, 6, 8, 10, 11 and 12, of the table on page 343, show traces of pale color on these parts. The markings at the tips of the wings are shown in Pl. XIV, Figs. 2, 4, and the dimensions of this brown area are given in the table just quoted. Dorsum of abdominal segment 10 with a median longitudinal carina; hind margin a little excised; appendages subequal to 10 in length, straight or nearly so, a little compressed, tapering to an acute apex, black. Ovipositor proper (gonapophyses of 8 + median gonapophyses of 9) reaching caudad not as far as the level of the hind end of 10, each lateral half having ten to twelve obliquely transverse ridges on its external (ectal) surface before the apex. Genital valves (lateral gonapophyses of 9) reaching caudad beyond the level of the hind end of 10, ventral edge of each in its caudal half hairy and denticulated, hind end emarginated, valvular process attached at the deepest point of the concavity and directed caudad and ventrad.

The variation in the number of antenodals is as follows, the numbers giving percentages of wings:

	14 <i>inopinata</i> ♂	14 <i>pellucida</i> ♂	12 <i>Th.</i> ♀
Front wings			
with 3	67.87	71.4	70.83
with 3 and rudimentary 4th	3.57	3.57	
with 4	28.57	25.0	25.0
with 2			4.16
Hind wings			
with 3	75.	71.4	62.5
with 3 and rudimentary 4th	3.57	7.1	
with 4	21.42	17.85	37.5
with 5		3.57	

In all cases the "rudimentary 4th" antenodal is a small stump projecting from the anterior side of the subcosta toward, but not reaching, the costa.

The relationships of *Thaumatoneura* have been discussed by McLachlan (1897), Needham (1903), Foerster (1909), and the writer (1902, 1913).

The larva of *Thaumatoneura* will be described in the next number of these studies.

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EXPLANATION OF PLATE XIV.

Figs. 1 and 2. Male *Thaumatoneura inopinata* and female taken pairing at Juan Vinas, Costa Rica, June 27, 1909. x $\frac{3}{4}$.

Figs. 3 and 4. Male *Th. pellucida* and female taken pairing at the same place, October 1, 1909. x $\frac{3}{4}$.

Fig. 5. Right hind wing of *Th. pellucida* ♂, shown in Fig. 3. x 2 1-6. All five figures from photographs by Mr. H. A. Walters.

THE MENDELIAN RATIO IN RELATION TO CERTAIN ORTHOPTERAN CHROMOSOMES

E. ELEANOR CAROTHERS

SIXTY-NINE FIGURES

CONTENTS

Introduction.....	487
Observations.....	489
1. Spermatogonia.....	489
2. Growth period and earlier diffuse stages.....	489
3. First spermatocytes.....	492
4. Second spermatocytes.....	496
5. Spermatids.....	497
Summary of observations.....	498
Discussion.....	498
Bibliography.....	504

INTRODUCTION

The aim of this paper is to describe the behavior of an unequal tetrad which occurs in the first spermatocytes of three members of the Oedipodinae: *Brachystola magna*, *Arphia simplex* and *Dissosteira carolina*. The distribution of the dyads of this tetrad, in relation to the accessory, follows the law of chance; and, therefore, affords direct cytological support of Mendel's laws. This distribution is easily traced on account of a very distinct difference in size of the dyads. Thus another link is added to the already long chain of evidence that the chromosomes are distinct morphological individuals continuous from generation to generation, and, as such, are the bearers of the hereditary qualities. McClung wrote ('05, p. 303), "In the absence, therefore, of definite knowledge of the chromosomes in the germ cells of organisms exhibiting Mendelian characters or mutations we are warranted in supposing them to be of the same general character as the ones

known till they are proved different." Since the above was written the increased knowledge of Mendelian phenomena has shown that they occur in practically every group of organisms. I hope that it may be possible to determine what the characters controlled by this particular pair of chromosomes are, through an experimental study of the living animals, correlated with a study of the developing soma of the embryo.

The credit for recognizing the importance of this tetrad belongs to Dr. C. E. McClung, as I was not sufficiently familiar with the ground to realize its general bearing when I found it. I am also indebted to him for consistent encouragement and guidance throughout the whole course of the work. The slides used were Dr. W. S. Sutton's, a few of Dr. McClung's and a number prepared by myself, from material in Dr. McClung's collection. In the work on the so-called nucleoli, or plasmasomes, I have been enabled to compare a number of different genera through the co-operation of D. H. Wenrich, a fellow student, who is giving especial attention to the growth period.

This work is based chiefly on *Brachystola magna*, a form already well known, so far as the general organization of the chromosomes is concerned, through the valuable researches of Sutton ('00, '02, '03). On this account, and in view of the fact that another paper by the same worker is soon to appear dealing with *Brachystola*, I shall restrict this paper strictly to the subject in hand, with the understanding that upon all other essential points my observations agree closely with those of Sutton.

While this chromosome does not come under McClung's original definition of a multiple, since it is not united with any of the others during the metaphase, it frequently forms a hexad during the prophase by uniting with one end of the accessory. It further separates itself from the ordinary chromosomes by having the spindle fibers attached to the free, instead of the synaptical ends, and in consequence, dividing transversely in the first spermatocyte when the other tetrads divide longitudinally.

OBSERVATIONS

1. *Spermatogonia*

Thanks to the accuracy of Sutton's drawings, but little remained in this stage for me to do. He found that the entire complex could be separated into two groups, one containing six small chromosomes and the other seventeen larger ones. Examination shows that his group of six small chromosomes is composed of five of about equal size and one decidedly larger. Two of his four spermatogonial figures show this clearly; in his first paper ('00), plate 33, figure 23, the largest chromosome of the small group, is apparently attached to the end of a member of the large group; in the second paper ('02) figure 2, the pair marked 'i' are clearly unequal in size. In figure 1 the chromosomes are present in oblique or end views, and in figure 3 the real size of either 'i' or 'j' may be concealed by the overlying chromosomes. To appreciate properly the weight of this evidence, we must remember that Sutton believed that they formed pairs, the individual members of which were of equal volume. This is true, probably, except for the two that unite to form the unequal tetrad. Figures 1, 3 and 4 of this paper show typical polar views of metaphases, with the entire group of twenty-three present, and reveal the relative volumes of the six small chromosomes clearly. Figure 2 shows the six small ones and their relative volumes, although it does not contain the entire complex.

Polar views of complete spermatogonial complexes of *Arphia* also show a possible separation into a group of small and a group of larger chromosomes, with the difference here that the small group consists of but four members while the larger one has nineteen. Close observation of the four small chromosomes shows that three of them are of practically equal volume and the remaining one (figs. 5 and 6, *a*) is slightly smaller.

2. *Growth period and earlier diffuse stages*

These observations are based principally upon *Arphia*, for while the nuclei of *Arphia* and *Brachystola* are practically identical in size those of *Arphia* are much clearer, partly owing to

the smaller size of the polar granules, described by Pinney ('08) for *Phrynotettix*. This point is especially in favor of *Arphia*, since part of the chromatin which produces the unequal tetrad passes through the greater part of the growth period, and other stages of general diffusion, in a dense condition where it appears as nothing less than one of the so-called plasmasomes or nucleoli so often described for these stages. In this condition it is liable to be confused with the ordinary large polar granules of *Brachystola*.

My earliest study of the growth period impressed me with the strong probability that these bodies which at certain stages stain like chromatin, are really composed of chromatin. Further study convinced me that at least one of them is associated with the unequal tetrad, and this conviction led to the present study.

Apical cells contain at least one, perhaps more, clear, straw-colored, more or less spherical vesicles. Close inspection reveals a minute, deeply staining granule at one point of the periphery; and further that this point is always in connection with a mass of chromatin (fig. 7, *k*). These latter, the peripheral granule and the connection through it with the ordinary chromatin, are absolutely characteristic features of such vesicles wherever they are found. Since they may or may not be filled with chromatin, the staining capacity is a variable factor.

At least two of these vesicles are present in the cells surrounding the apical cell (fig. 8, *k*). In favorable preparations they may be traced in the several spermatogonial generations from early telophases (fig. 9 *k*) to later prophase (fig. 10 *k*). The one represented in figure 10 is connected to a chromatin thread with unequal arms, and, as telosynapsis of the elements which form the unequal tetrad of the first spermatocytes occurs at a stage even preceding this, as is clearly shown by figure 11, *c*, there is little doubt that this, also, is the precocious tetrad and its associated vesicle.

During the growth period there are three vesicles; two single and one double (fig. 13, *k, k, k*) (occasionally, one-half of the latter splits again forming a tripartite vesicle). For this reason, and especially since no thorough study of the earlier stages was undertaken, the largest number found at that period—two—

should not be taken as established. In early bouquet stages the vesicles are colorless and always occur on the distal part of their respective loops—that is, at the point farthest from the center of radiation of the chromatin threads. Figure 12, *k*, shows the double vesicle and its associated loop occupying a position in relation to the accessory, *x*, which is characteristic for them at this stage. The single vesicles behave in a similar manner; that is, they are colorless, except for a dense peripheral granule through which passes the spireme thread. Very soon all of the vesicles become densely stained (with Flemming's tricolor, iron hematoxylin or Auerbach's stains) but are still certainly recognizable on account of the granule which can yet often be made out. The halves of the double vesicle come to lie opposite each other, separated only by a dense granule common to both, through which the chromatin thread passes (fig. 14, *k*). This thread is thinner than any of the others and even a fragment of it separated from the vesicles can be identified. What the later history of this chromosome is has not yet been worked out. One of the single vesicles is associated with the unequal tetrad, as later evidence shows. Near the end of the growth period they begin very gradually to lose their staining power (fig. 15) until finally their outline becomes so faint that they can no longer be distinguished.

Figure 16 represents the latest stage of the growth period in which they are easily apparent in *Arphia*, though in some forms they persist till a much later period. Figure 23, *k*, shows one in *Brachystola*, associated with the unequal tetrad and persisting until a late prophase. Figure 29, *k*, shows the same vesicle still recognizable at the first spermatocyte metaphase. Since the plates were finished, favorable preparations have shown them in the second spermatocyte anaphases and in the spermatids. The term 'plasmasome' is self-evidently not applicable to these bodies, since their content is chromatin and they are distinctly concerned in the formation of the chromosomes. Winiwarter indicates ('12, fig. 25) a similar condition in human spermatogenesis. His drawing shows the peripheral granule and its connection with the spireme beautifully, although he does not mention either fact and calls the body a nucleolus.

Figure 11 represents an early secondary spermatogonium and is of especial interest on account of the loop shown at *c*. The dense granules at both ends of the dyads are very evident. This is a peculiarity that marks certain chromosomes as was noted by Miss Pinney ('08, p. 313). The curve of the free and of the longer dyad of the unequal tetrad (fig. 19 *c*) had long puzzled me, but in view of the earlier relation of these elements shown in figure 11 *c* it is evidently due to the separation, at this point, of the dyads which had previously been united at both ends. During the growth period this tetrad is associated with the accessory both in *Arphia* and *Brachystola*. This is probably merely a persistence of the conditions established at the formation of the composite granule. When the accessory breaks away from this body it carries with it several granules with their chromatin threads¹—in *Brachystola*, for a time, forming a conspicuous second center of radiation. Figure 25 shows all three small chromosomes still adhering to it, but the relation with the unequal tetrad is the most persistent. Figures 18, 18 *e* and 19 are successive stages in *Arphia*. Figure 17 is from *Brachystola*.

3. First spermatocytes

Brachystola. As soon as the chromosomes have become sufficiently condensed to be recognized as distinct individuals, the accessory, which at first is in the form of a 'U' with the arms approximated, is often seen connected with a small chromosome more dense than any of the others, except the accessory itself (fig. 24). There are at this time eleven individuals, counting the multiple as one (fig. 28). The larger dyad of this tetrad shows a transverse constriction, giving the whole a tripartite appearance. Either the larger or smaller end may be attached to the accessory (figs. 23 and 24).

In early metaphases the chromosomes appear as twelve separate individuals. Side views show the accessory in its characteristic position near one pole. The smallest chromosome frequently shows a constriction; the next in size rarely gives an indication of approaching division, while the third is entirely separated, only a

¹This was first noted by Wenrich who also suggested the term 'composite granule.'

thin thread joining the two parts. A decided difference in the size of the two dyads proclaims this as the tetrad formerly united to the accessory.

While all the other tetrads are made up of quantitatively equal parts and follow the typical Orthopteran plan of division (longitudinal in the first spermatocyte and transverse in the second) already worked out for *Brachystola* by Sutton, this tetrad divides transversely in the first spermatocyte, as is evident from an examination of the prophases, where it is united with the accessory so that the longitudinal split between the chromatids of the tetrad corresponds with the division between the chromatids of the accessory. This involves the further exception that the spindle fibres must become attached to the free instead of the synaptical ends. The unequal size of the two dyads allows no mistake on this score; otherwise, we should have one large and one small chromatid passing to each second spermatocyte (a condition which Wenrich has actually found in a nearly related genus, *Phrynotettix*). Here we see that although the connection with the accessory is lost, this tetrad is still in the line of behavior described by McClung ('05) for the multiples of *Hesperotettix* and *Anabrus*, which remain associated with the accessory throughout the maturation divisions and are made up of quantitatively equal parts.

Three hundred cells were drawn under the camera lucida to determine the distribution of these dyads in relation to the accessory. Of these, 228 show the accessory and tetrad in the same section, and as this does not include any case in which there is any uncertainty, either in regard to the pole for which the accessory is destined or where there is the possibility that the dyads do not reveal their true size, there can, I think, be no reasonable doubt of the results. In 107 cells the smaller dyad was going to the same pole as the accessory, and in the remaining 121 the larger dyad occupies this position. In the other 72 the accessory and tetrad are in different sections, but great care was used to make sure that there was no mistake in identifying the cell or in labeling the drawings. The smaller is accompanying the accessory in 39 of the cells, and the larger in 33. This practically agrees with

the result in the case where no confusion was possible, owing to both accessory and tetrad appearing in the same section. As a net result, then, in the 300 cells drawn, the smaller dyad would have gone into the same second spermatocyte as the accessory, 146 times, or in 48.6 per cent of the cases; and the larger one, 154 times, or in 51.3 per cent of the cases. I might further state that 32 cells were drawn from one individual and a larger number from another, each giving substantially the above results, before I saw the trend of the evidence.

Second spermatocyte figures are not striking in my material on account of the lesser difference in volume of the dyads, but early anaphases of the first spermatocyte, such as figures 33, 34, 41, 42, 45, 49 and 57 where the attachment of the spindle fibers is clearly evident, speak for themselves. The majority of my drawings are of early metaphases as represented in figures 30 to 32, 37 to 40, and so forth, but with a number of later figures to substantiate them, there seems to be no reason to doubt their reliability. More conclusive still, are polar views of late anaphases (figs. 58, 61 to 64). Figures 61 and 63 contain twelve chromosomes, including the accessory; in figure 63 one of the three small dyads is markedly larger than the other two—the larger dyad; while in figure 61 one is slightly smaller than the other two—the small dyad. Figures 58, 62 and 64 are eleven chromosome groups, the first two containing the larger, the last the smaller dyad.

One peculiarity is yet to be noted. In many instances the larger dyad has a constriction about one-third nearer the proximal than the distal end (fig. 57) corresponding to the tripartite appearance of the prophase. Usually it is very slight; sometimes no indication of it can be found, while in a few instances it is carried to the extreme seen in figure 29. Apparently this is an individual variation.

The unequal pair is comparable to any of the other tetrads and is not part of a sex group, such as has been described by Payne ('09), since the distribution of its parts is not related to sex, as indicated by the presence or absence of the accessory. As a corollary, it follows that its arrangement on the spindle is a matter

of chance. This is the first demonstrable case, so far as I know, showing that the maternal and paternal chromosomes do not pass collectively to given poles.

Payne, from his work on *Gryllotalpa borealis* Burm ('12) where he reports a large accessory and an unequal pair, the larger member of which always passes to the second spermatocyte which receives the accessory, argues that there is no haphazard arrangement, as is necessary for the explanation of Mendelian phenomena, that the chromosomes brought into the male by the egg pass into the female producing spermatozoon. A few lines later, in order to explain the transmission of characters from father to daughter, he says that a satisfactory explanation is found in the synaptic stage, assuming an interchange of the smaller units that make up the chromosomes. So, after all, he would not have the *material* (chromatin), which is the essential thing contributed to the male by the egg, pass into the female determining spermatozoon. Besides, as I shall point out more fully later, (p. 503) an interchange of material during synapsis could only affect the second generation and not the immediate offspring, though that is what he is attempting to explain.

Arphia. In early prophases the accessory is in the form of a U and well condensed (fig. 19*x*), and the unequal tetrad is nearly as dense as the accessory, the longer dyad having a curve at the free end as though it had been drawn over towards the free end of the shorter (fig. 19*c*). One of the large tetrads is only slightly less precocious; a fragment of this chromosome is shown at *b*, figure 19. In late prophases the unequal tetrad has become straight and the accessory is but slightly curved (fig. 26). They are occasionally found associated end to end—a continuation of the growth period relation.

Twenty-five side views of metaphases and anaphases were drawn (figs. 52, 54, 55 and 56). Twelve contain the accessory and the larger dyad on the same side of the equatorial plate, and thirteen the accessory and smaller dyad in the same relation.

The complex differs from that of *Brachystola*, as may be seen by a comparison of figures 32, *Brachystola*, and 35, *Arphia*; (the latter lacks the accessory but contains the remainder of the com-

plex). The important point is that there are two small chromosomes in *Arphia*, instead of three as in *Brachystola*, and that one of these divides equally while the other divides unequally. These two small tetrads are very nearly the same size and might easily lead a hasty observer who was not familiar with both tetrads to the conclusion that they are homologous chromosomes when he observed them separated as they often are in sectioning and he would repeat the statement frequently seen that one of the small chromosomes sometimes divides unequally. The fact is that one particular small chromosome always divides unequally in first spermatocyte metaphases of *Arphia* simplex.

No indication of a secondary constriction of the larger dyad has been observed.

Dissosteira carolina. Figures 47, 53, 59 and 60 are typical first spermatocyte views. The larger dyad is constricted as represented (fig. 53), in numerous cases. In this genus it will be noticed, however, that the constriction is about the center instead of nearer the proximal end, as in *Brachystola*. One of the large tetrads shows a weakness in one chromatid of each dyad, as seen in figure 59. This has been found in several animals, and appears characteristic of that chromosome, though it does not always occur; in this way resembling the constriction found in the larger dyad of the unequal tetrad. That this peculiarity marks certain chromosomes and always occurs at the same point when distinguishable, is further evidence of the precise arrangement of their constituents, whether we consider it to be due to chemical, electrical or mechanical forces. A suggestive discussion of this subject is given by Agar ('12).

4. *Second spermatocyte*

Only a very brief period ensues between the first and the second spermatocyte divisions, but when the chromosomes become arranged in the equatorial plate, one of the small chromosomes is again sometimes found associated with the accessory, and, as one would expect from a knowledge of the first spermatocytes, varies in size, depending, evidently, upon whether the larger or

smaller dyad accompanied the accessory. Owing to the decreased size of all the chromosomes at this stage, the difference in volume of the smaller chromosomes is difficult to distinguish, and to be worth anything the figures compared must be not only from the same animal, but from the same slide, in order to avoid complications due to fixation and staining. Figures 65 to 68 comply with these conditions.

The result is clearly four sorts of spermatozoa. One-half contain the accessory, and of these again one-half contain one of the larger chromatids and one-half one of the smaller. Likewise, the spermatozoa without the accessory may be classed as those containing the large and those containing the small chromosome.

5. *Spermatids*

Spermatids of *Arphia simplex*, at the time when the ordinary chromatin has become quite diffuse, contain the three condensed elements; accessory, large precocious chromosome and one member of the unequal pair still in a dense condition. Figures 21 and 22 are drawings from such a stage of twelve chromosome spermatids, with the accessory, of course, present. One contains the large and one the small chromosome, *c.* A comparison of some of the features of these two spermatid nuclei with the nucleus at the end of the growth period shows the volume to be reduced about one-fourth. This, of course, is what might be expected, since four cells have been formed from the one with practically no resting period between the two divisions. The accessory is approximately one-half the volume of the accessory of the first spermatocyte which has undergone but one division (figs. 54 and 55). The large precocious element is beginning to diffuse, hence is more than one-half the size of the first spermatocyte dyad from which it is derived. The same applies to the derivative of the unequal tetrad.

SUMMARY OF OBSERVATIONS

1. The so-called plasmasomes or nucleoli are always associated with certain spireme threads or prophase chromosomes; in these species they never exist as free bodies. This connection is through a peripheral, dense granule.

2. During the growth period there are three of these vesicles, two single and one double.

3. They occur at the distal end; that is, opposite the center of convergence of the chromatin threads of the loops formed by the union of the spermatogonial chromosomes.

4. They have been found at all stages in the history of the germ cells, being visible even in the first spermatocyte metaphase, second spermatocyte telophase, and in the spermatids.

5. Their staining power varies; but in general they stain most densely when the chromatin is most diffuse and give up this power as the chromosomes condense.

6. The first maturation division is longitudinal for all the ordinary tetrads. This is proven by a knowledge of their structure gained from the prophase, by the point of attachment of the spindle fibers, and by the appearance of the rings during division.

7. One of the tetrads which is associated with the accessory chromosome during the growth period divides transversely in the first spermatocytes and longitudinally in the second, which the accessory also does in effect.

8. The dyads of this tetrad are unequal in size, hence the chromosome is easily recognized.

9. The different parts of this tetrad are distributed equally to both sorts of spermatozoa.

DISCUSSION

Sutton ('02) showed that the spermatogonial chromosomes occur in pairs, and his conclusion was that in the somatic cells and in the spermatogonia there is a double series composed of homologous maternal and paternal chromosomes. This theory was put forward by Boveri ('01) but it remained for Sutton's work to furnish direct evidence. These homologous pairs unite

in synopsis, and in the reduction division are separated into groups which are neither purely paternal nor purely maternal. This last suggestion, I believe, was pure theory advanced to meet the known experimental facts which show that either parent may transmit the characters of its ancestors of the opposite sex. It is strange that so careful an observer should have overlooked the very thing that was present (the unequal tetrad was first found on Sutton's slides) offering definite chromosomic proof for his theory. For, I think, there can be little doubt that the dyads of the tetrad described in the foregoing pages are distinct physiological individuals, representing respectively the paternal and maternal contribution to the formation of some character or characters; and, as each can be identified, they furnish an excellent means of tracing the process of segregation and recombination.

None of the female germ cells in maturation stages being available at present, definite knowledge of what occurs there is lacking. But it seems necessary to assume the presence of an unequal tetrad there also, for if its place were filled by an ordinary chromosome of equal parts we would sometimes find two large or two small united in the first spermatocyte, but in every one of the twenty animals studied the unequal tetrad was present. The only alternative would be to conclude that one-half the spermatozoa are not functional, for which there is not a shred of evidence.

If this assumption that after maturation one-half the ova contain the large and one-half the small dyad be correct, then selective fertilization becomes a necessity, since a spermatozoon containing the large dyad could only fertilize an ovum containing the small. This would in no way interfere with the ratio of Mendelian characters, for, owing to the abundance of spermatozoa, all the ova would be fertilized; nor does it necessitate the extension of the theory to those pairs which are quantitatively equivalent. But, whether further evidence shall show selective fertilization to be a fact or not, and regardless of the mode of origin of the inequality, its absolutely constant occurrence and the alternate distribution of the dyads in even one individual is sufficient for the essential part of this work—the segregation of at least part

of the paternal and maternal chromatin according to the law of chance.

The large dyad, which I shall arbitrarily designate as bearing characters of the male line, even when transmitted by the female, must represent characters of her male ancestors, either on the paternal or the maternal side. In like manner, the small dyad must represent characters of the female line, though half the time contributed by the male and, consequently, bringing in characters of his female ancestors. In other words, so far as this one pair of chromosomes is concerned, the spermatozoa may contain the small chromosome carrying factors from the maternal grandmother or from the paternal grandmother, or it may contain the large chromosome carrying characters of the paternal grandfather or the maternal grandfather. Likewise, the ova after maturation may contain, by hypothesis, the large chromosome inherited from either the paternal or the maternal male lines, or the small from the maternal or the paternal female lines. Eight combinations are, then, possible for this pair, as shown in the accompanying diagram. The possible combinations with various numbers of chromosomes has been worked out by Sutton ('03, p. 234, et seq.), so that further work on this line is superfluous. However, it may be well to emphasize the fact that the 68,719, 476,736 combinations which he has shown to be possible in the zygotes of organisms possessing 36 chromosomes in the somatic series is amply sufficient to account for all observed variations, without the assumption of any interchange of material between the chromosomes.

The only question that remains to be considered is whether Mendelian phenomena are exceptional cases of heredity, or whether they represent the type form of all inheritance. Perhaps the strongest arguments against the latter view have been derived from blends, first crosses that breed true and mosaics.

In regard to the first, Hatai ('11) has shown that the series obtained from the square of the binomial $(X^2 + 2XY + Y^2)^n$ expresses the distribution of determinates for both Mendelian and blended inheritance, and that therefore the latter may be

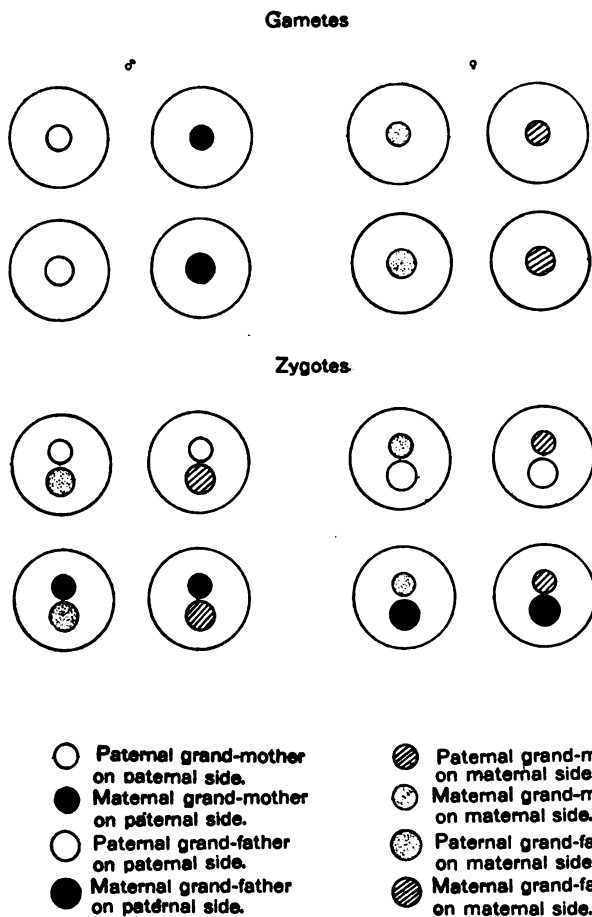


Diagram of Possible Combinations of Unequal Pair

considered as a limiting case of Mendelian inheritance where dominance is imperfect. In what are commonly recognized as cases of Mendelian inheritance the hybrid is indistinguishable from the dominant homozygote so far as gross characters are concerned, and the only means of sorting the pure from the hybrid offspring is by inter-breeding. But this is not necessarily the case, as shown by Punnett ('07, pp. 29-30). In the Leghorn breed of poultry white plumage is dominant to colored, but not perfectly so. To quote directly:

When a white and a brown Leghorn are crossed together, all the resulting offspring are white, but almost invariably have a few colored feathers. The presence of these 'ticks' is the outward and visible sign of the heterozygous nature of the bird on which they occur. Such birds give off equal numbers of gametes bearing the white and colored characters. This is easily tested by breeding them together. It is found that from such matings one-quarter of the offspring are colored recessives, whilst the remainder are pure white, or white with a few ticks. The heterozygote resembles the dominant form much more closely than it does the recessive. Though we may speak of dominance in such a case it is necessary to remember the dominance is not perfect. This, however, makes no difference to the essential feature of Mendel's discovery, which is, of course, the segregation in the gametes of the factors corresponding to the dominant and recessive characters.

This differs only in degree from typical cases of blended inheritance. Yet it is clearly Mendelian, and accords perfectly with Hatai's idea of incomplete dominance. The segregation of pure colors in the gametes proves that there has been no interaction affecting the essential character of the determinants.

A case of complete blending in the first generation, followed by segregation in the second, is given by Castle ('11, p. 138). This is in regard to length of ear in maize, and has been worked out by East.

As to first crosses that breed true, it seems difficult to find such cases. The mulatto has been cited; but Davenport, handling the matter in a scientific manner, finds that there is segregation in the ratio of one to sixteen which can be brought into harmony with other Mendelian results by the assumption of four factors for black in the negro.

In regard to mosaics, Sutton says in part ('03, p. 245) "If each cell contains paternal and maternal potentialities in regard to each character, and if dominance is not a common function of one of these, there is nothing to show why as a result of some disturbing factor one body of chromatin may not be called into activity in one group of cells and its homologue in another." This view is supported by blends which later segregate out pure characters as well as the work of Tennent ('10) reversing the dominance in Echinoderm hybrids by changing the concentration of the hydroxylions in the sea-water in which they developed.

A consideration of the limited number of chromosomes and the large number of characters in any animal or plant, will make it evident that each chromosome must control numerous allelomorphs, or unit characters. It is to the individual dominance, either partial or complete of these unit characters, rather than to the dominance of the chromosome as a whole, that we may look for the explanation of Mendel's laws.

Since the rediscovery of Mendel's laws, increased knowledge has been constantly bringing into line facts that at first seemed utterly incompatible with them. There is no cytological explanation of any other form of inheritance; the long association of synaptic pairs during the growth period has suggested the possibility of some interaction between the chromosomes, but this association is between the chromosomes of the grandparents, directly. Let us suppose that these represent pure lines on both paternal and maternal sides, and that the character under consideration is a blended one. Now, the fertilized ovum resulting from a cross between these two pure lines at once develops an organism with the blended character without any long association of the chromosomes that produce it. In other words, if there were an interaction between chromosomes during the growth period, which would result in blended inheritance, it could not be manifested until the second hybrid generation. It seems to me probable that all inheritance is, in reality, Mendelian.²

² All observational work was done at the University of Kansas. Some drawings have been completed and the paper revised at the University of Pennsylvania.

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EXPLANATION OF PLATES

All figures are from camera lucida outlines. Original magnification 2400 diameters. Actual magnification of figures 1200 diameters.

Numbers 21 and 22, spermatids, are displaced from the position they would occupy in order of development for purposes of comparison; the others are arranged as nearly as convenient in that order.

All drawings are from *B. magna* unless otherwise indicated. Ordinary chromosomes are in outline for clearness of comparison.

Accessory at upper pole except figures 43 and 55.

PLATE 1

EXPLANATION OF FIGURES

1 to 4 Spermatogonial complexes of *Brachystola magna* showing division of entire complex into two groups; one containing six smaller chromosomes, the other seventeen larger ones. Figure 2 does not contain all of the larger chromosomes but shows the accessory, *x*, in recognizable form. Of the smaller group the probable pairs are designated *a*, *b*, and *c*, respectively. One of those marked *c* is obviously larger than any of the other five.

5 to 6 Spermatogonial complexes of *Arphia simplex*. Two groups with regard to size are again evident with the difference that there are four smaller and nineteen larger chromosomes. Three members of smaller group are practically identical in size; the fourth, *a*, is smaller.

7 Apical cell of *Arphia simplex*. Persistent chromatin vesicle *k* associated with flocculent mass of chromatin.

8 Nucleus from one of the cells surrounding apical cell, containing two persistent chromatin vesicles *k*, *k* with their associated chromatin masses.

9 Spermatogonial telophase of *Arphia simplex* showing vesicles *k*, *k* and associated chromatin threads.

10 Spermatogonial prophase *Arphia simplex*. One end of associated chromatin thread longer than the other.

11 Spermatogonial spireme, *Arphia simplex*, *c*, telosynaptic union of unequal pair; *x*, accessory; *p*, polar granules.

12 Early growth spireme, *Arphia simplex*. Position of persistent chromatin vesicle, *k*, on loop and relation of this loop to accessory, *x*, typical.

13 Same as above at a slightly later period as shown by splitting of threads. Chromatin in vesicles *k*, *k*, forming definite chromatin bodies.

14 Fragment of nucleus, *Arphia simplex*. Chromatin bodies *k* still densely staining, associated thread thinner than ordinary threads.

15 Very slightly later stage, *Arphia simplex*. Bodies *k*, *k* less densely staining.

16 Still later stage, *Arphia simplex*. Vesicle *k* persisting though nearly colorless. Associated thread more dense than ordinary threads.

17 Part of cell of *Brachystola magna* near end of bouquet stage; *x*, accessory with precocious tetrad attached.

18 Part of cell *Arphia simplex* near end of growth period; *x*, accessory; *c*, unequal precocious tetrad attached to accessory. 18 e, *x* and *c*, later stage.

19 The same; early prophase; *x*, accessory; *c*, unequal precocious tetrad; *b*, fragment of another precocious tetrad.

20 Late prophase of *Brachystola magna*. Homogeneous accessory and associated precocious tetrad showing tripartite feature and greater density than ordinary tetrads.

21 Spermatid, *Arphia simplex*. The three condensed elements still intact; *x*, one chromatid of accessory; *b*, one chromatid of larger precocious tetrad; *c*, one of smaller chromatids of unequal tetrad.

22 The same except *c*, one of larger chromatids of unequal tetrad. Note that the nucleus of either spermatid is about $\frac{1}{2}$ that of the nucleus at end of growth period.

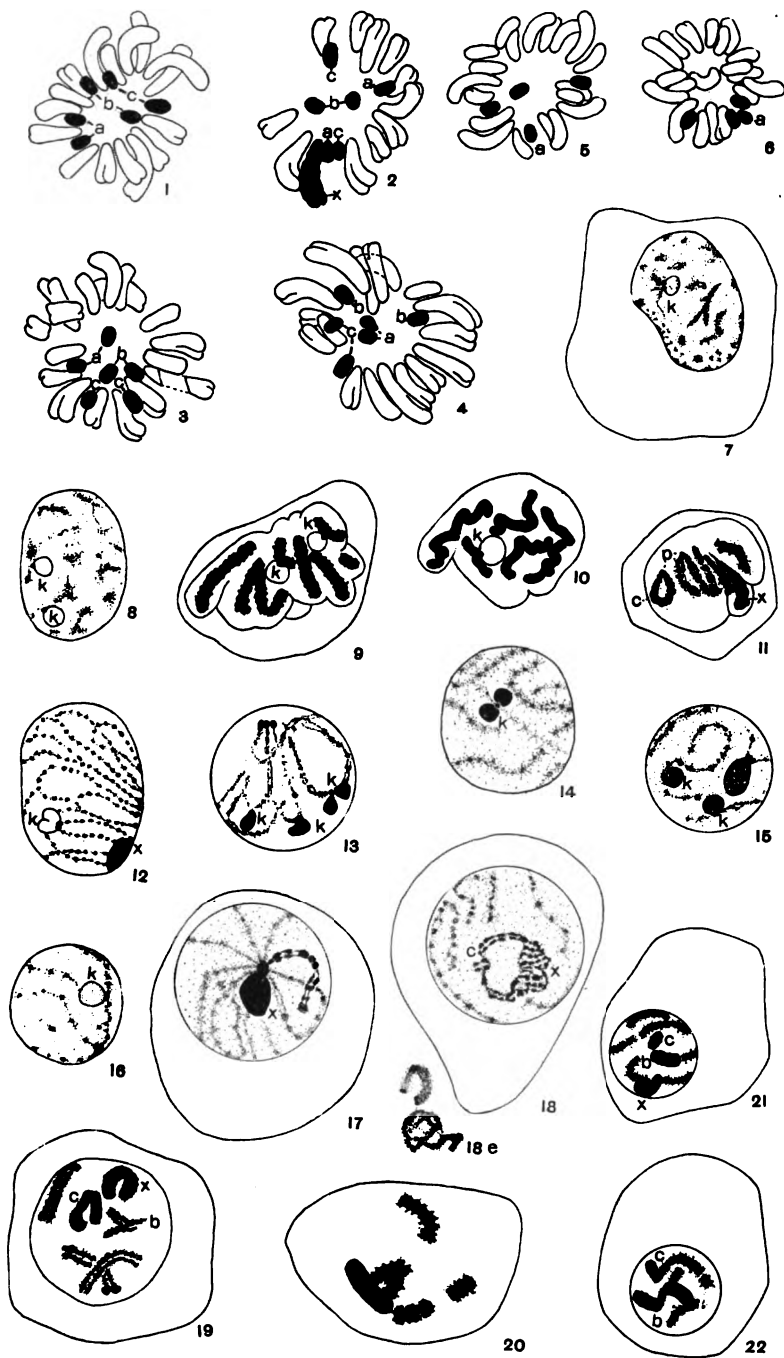


PLATE 2

EXPLANATION OF FIGURES

- 23 Accessory and unequal tetrad forming a hexad multiple; *k*, remains of vesicle, smaller dyad attached to accessory.
- 24 The same; larger dyad attached to both ends of U-shaped accessory.
- 25 The same; all three small tetrads adhering to accessory.
- 26 Late prophase, *Arphia simplex*; unequal tetrad separate from accessory.
- 27 First spermatocyte metaphase; (this and most of the remaining figures are designed to show the distribution of the unequal tetrads in relation to the accessory).
- 28 Late prophase of entire first spermatocyte complex: at this time there are eleven separate elements; drawn from three sections.
- 29 Unequal tetrad with persistent vesicle, *k*, still distinguishable at first spermatocyte metaphase.
- 30 First spermatocyte metaphase from same animal as figure 39. Note that in the first instance the larger and in the last the smaller dyad accompanies the accessory.
- 31 and 32 Entire first spermatocyte complexes: 31 drawn from two sections, 32 from three.
- 33 and 34 Early anaphases of entire complexes. In figure 33 the smaller dyad accompanies the accessory while in figure 34 it is the larger one. Both drawn from two sections.
- 35 Entire complex of *Arphia simplex* except accessory. Note that there are only two small tetrads here corresponding to the four small chromosomes of the spermatogonial group; figures 5 and 6. Also note the general differences between the complexes of the two genera by comparing with figures 31 and 32.
- 36 First spermatocyte metaphase from same animal as figure 32.
- 37 and 38 First spermatocyte metaphases from another animal.
- 39 and 40 The same from a different animal.
- 41 and 42 Anaphases from still another animal illustrating the same feature.
- 43 and 44 From same animal as figures 39 and 40.

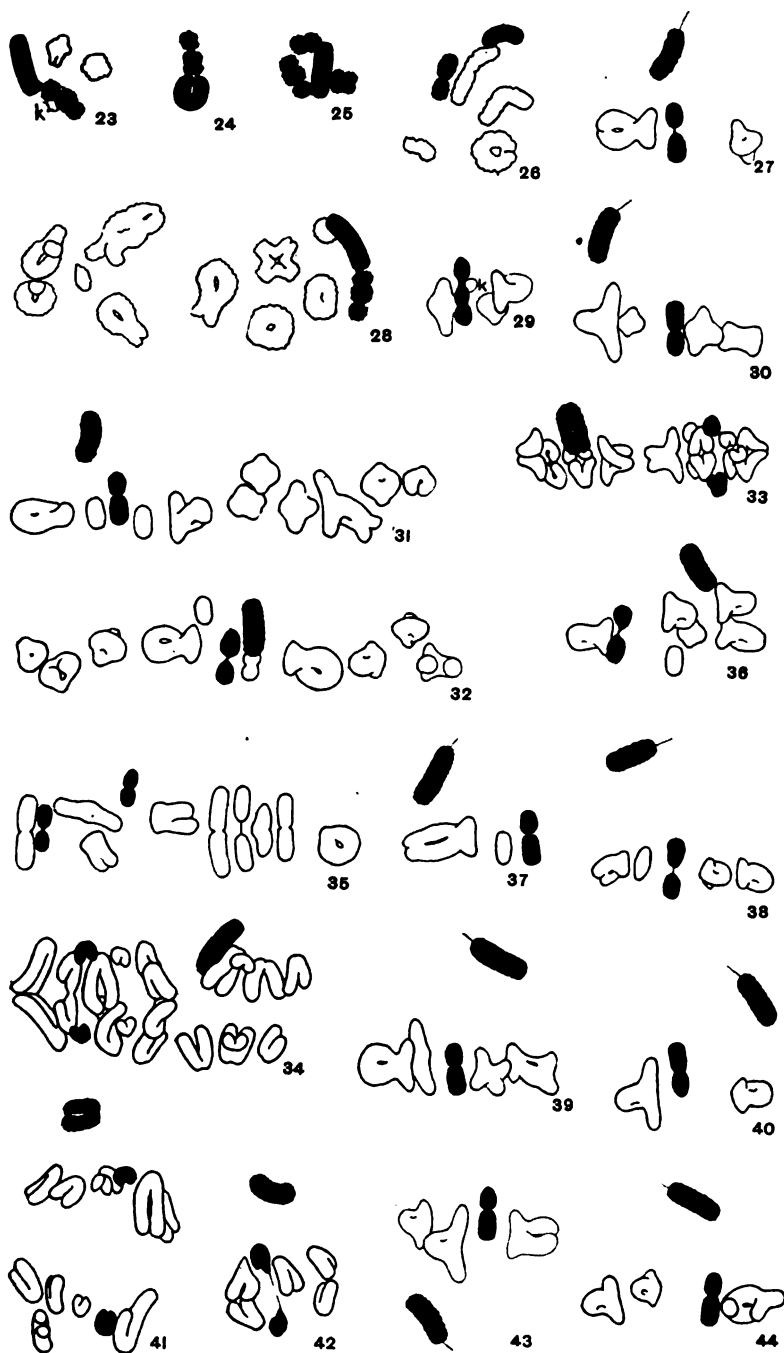
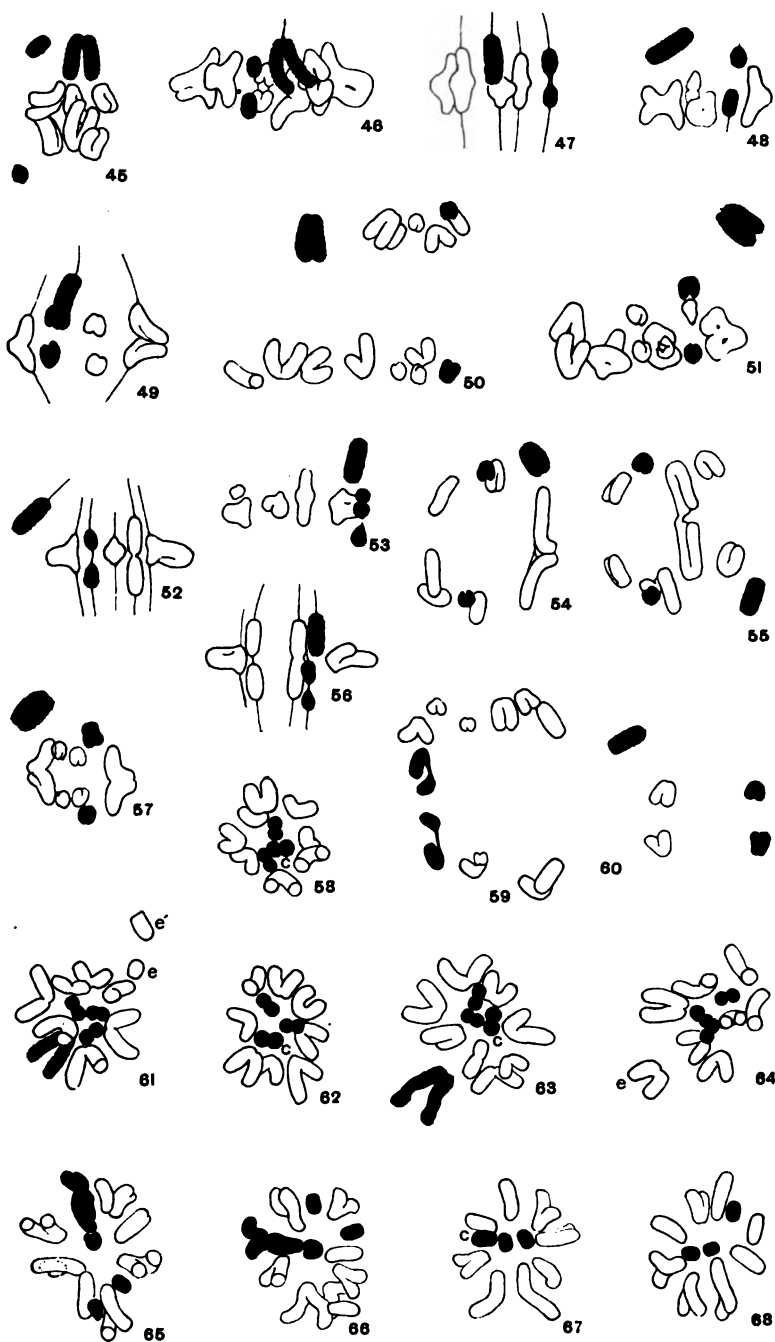


PLATE 3

EXPLANATION OF FIGURES

- 45 Early first spermatocyte anaphase from same animal as figures 48, 50 and 57.
- 46 Late metaphase from yet another animal.
- 47 Metaphase from *Dissosteira carolina*.
- 48 Metaphase from same animal as figure 45.
- 49 Late metaphase or early anaphase from same individual as figure 34.
- 50 Late anaphase.
- 51 Metaphase from another animal.
- 52 Metaphase from *Arphia simplex*.
- 53 Metaphase from *Dissosteira carolina*; note secondary constriction of larger dyad.
- 54 and 55 Late anaphases from *Arphia simplex*.
- 56 Metaphase from *Arphia simplex*.
- 57 Late metaphases from same animal as figure 48. Note tendency to constriction of larger dyad.
- 58 First spermatocyte telophase, eleven chromosome group containing the larger dyad, *c*, and lacking accessory. (All telophases are not only from the same individual but also from the same slide.)
- 59 Late anaphase *Dissosteira carolina* showing weakness of one dyad of one of larger chromosomes.
- 60 Anaphase of *Dissosteira carolina* from same animal as figure 47.
- 61 Telophase of twelve chromosome group. Accessory and smaller dyad present; *e*, fragment of *e'* from another section.
- 62 Similar to figure 58.
- 63 Similar to figure 61 except that larger dyad, *c*, is present instead of smaller one. Accessory drawn from another section.
- 64 Similar to figures 58 and 62 except that it contains smaller instead of larger dyad; *e*, ordinary dyad from another section.
- 65 and 66 Second spermatocyte metaphases containing accessory; and figure 65 smaller dyad, figure 66 the larger one.
- 67 and 68 The same as above, eleven chromosome groups, one containing the larger dyad, *c*, the other the smaller.



Two new *Otostigmi* from India (*Myriop.*).

By RALPH V. CHAMBERLIN, University of Pennsylvania,
Philadelphia.

The two new species of *Otostigmus* here described may readily be distinguished from the other known species of the genus bearing spines upon the prefemur of the anal legs by means of the following key. In this key only the more closely related forms are actually taken up; but it is constructed so as to eliminate all others.

- a. Dorsal plates margined from the 21st segment cephalad at least to the eighth.
- b. The three proximal articles of the antennae glabrous or nearly so.
- c. Ventral plates without true longitudinal sulci; at most with shallow furrows or pit-like depressions.
- d. Last ventral plate with the sides parallel and the caudal margin convexly rounded; ventral plates with eight impressions.
O. cuneiventris Poc.
- dd. Last ventral plate with sides converging caudad and the caudal margin straight or incurved.
- e. Prefemur of anal legs without a corner spine at distal end above; ventral plates with two median depressions, one caudad of the other *O. ceylonicus* Haase
- ee. Prefemur of anal legs with a well developed corner spine; ventral plates with a pair of depressions lying one each side of median line and a small median depression at caudal margin *O. simplex* sp. nov.
- bb. Only the 2 1-3 to 2 1-2 proximal articles of the antennae glabrous.
- c. Dorsal plates, excepting at most the first seven, bearing from 7 to 9 longitudinal keels which are serrate or tuberculate.
- d. At most the first 7 pairs of legs with two tarsal spines, the others with 1, or 20th and 21st with none.
- e. Articles of antennae 21; sulci of sternites weak, developed only on anterior 1-3 or 1-2 of plate; 20th legs with no tarsal spine.
O. scaber Pocock.
- ee. Articles of antennae 17; sulci of sternites strongly developed, crossing the entire plate; 20th legs with a tarsal spine.
O. amballae sp. nov.
- dd. First 18 pairs of legs with two tarsal spines. Head and first dorsal plate chestnut, with the other dorsal plates bluish green *O. insularis* Haase

Otostigmus amballae sp. nov.

Dorsum brown, olive along middle part of caudal border and in a number of interrupted or broken longitudinal lines or streaks. Head and first dorsal plate somewhat paler than dorsum. Legs testaceous or yellowish; the posterior pairs distinctly ringed with bands of green.

Head shining; showing numerous fine punctae with fewer coarse ones intermixed.

Antennae composed of 17 articles of which the first two are practically glabrous and the third one similarly so at proximal end.

Dorsal plates from the third to the penult inclusive, longitudinally bisulcate, the sulci deep and completely crossing the plates. Plates from about the fifth caudad distinctly margined. All plates excepting the first two and the last one with a low median keel, one each side of this geminated by the sulcus, and two between the sulcus and the lateral margin, these more lateral keels being more or less broken and bearing inconspicuous tubercles along edges; keels most distinct on the caudal segments. First and second plates smooth. All plates punctate. Last plate simply bowed out caudad, the mesal portion of margin rounded, not angular; with a median longitudinal keel extending from the anterior margin caudad a little more than half the length, this keel being followed on the caudal portion of the plate by a conspicuous median furrow; lateral portions of plate smooth or nearly so.

Prosternal teeth 4 + 4; of these the most ectal is much the smallest and the second from mesal line much the largest, the other two being of intermediate size; line of apices of teeth strongly convex; all teeth angular.

Ventral plates from the second to the penult inclusive, deeply longitudinally bisulcate; also with a wider and shallower median longitudinal furrow which is deepest in front of middle. Plates finely punctate and those of caudal half of body tuberculate; the tuberculation becoming more and more conspicuous caudad.

Last ventral plate narrowed caudad, with the sides convex and the caudal margin weakly concave. On caudal half with a distinct median longitudinal furrow which continues cephalad as a pair of finer and less distinct furrows.

Processes of coxopleurae rather long, extending caudad of ventral plate about two-thirds the length of the latter; terminating in four spines or points, and also with a fifth spine on coxopleural margin near caudoectal corner.

At least the first three pairs of legs with two tarsal spines, the others, or all but a few succeeding these, to the twentieth inclusive, with one tarsal spine.

Prefemur of anal legs armed ventrally with an ectal series of 3 spines and a mesal one of 2; in addition with a strictly mesal series of 3 spines increasing in size distad, and along dorsomesal surface a series also of 3 spines, the distal one of the latter being the corner spine at end of joint. Other articles unarmed and smooth.

Length, 38 mm.

Locality.—Amballa, India (Rev. M. M. Carleton coll., 1873). The type is in the Mus. of Comp. Zoology at Cambridge.

Otostigmus simplex sp. nov.

Type specimens at present much bleached from long preservation so that original coloration cannot be definitely ascertained. At present they are brownish, with a pale median longitudinal stripe. Legs yellowish.

Head finely and sub-densely punctate, the punctae being more sparse in the frontal region. A weak median longitudinal furrow midway between sulcus and caudal margin and a more sulciform impression from anterior margin a short distance caudad.

Antennae composed of 18 articles of which the first three are glabrous.

Dorsal plates from the fourth to the penult longitudinally bisulcate. Plates from the 7th or 8th caudad distinctly laterally margined. Finely punctate, but otherwise very smooth, showing no carinae, or at most on some of the caudal plates with a median one obscurely set off by 2 faint longitudinal furrows.

Last dorsal plate with caudal extension sub-triangular, the lateral portions straight or nearly so from corners to median line, the median angle narrowly rounded.

Prosternal teeth 5 + 5; all teeth well rounded and the more mesal ones appearing as mere crenations in the dental plate, the ectal ones better separated and more angular. Process of femuroid of prehensors large; with a single rounded crenulation or tooth on ectal side and two on the mesal.

Ventral plates all finely punctate. Wholly lacking true sulci; but each with a broad longitudinal depression or furrow on each side which does not reach either the anterior or the posterior margin; in addition there is a much smaller median foveola or depression immediately in front of the caudal margin.

Last ventral plate narrowed caudad, the converging sides straight, excepting at ends, where they are convex, caudal margin distinctly incurved. Convex, smooth and without furrows, excepting for a median longitudinal one over the caudal third of length.

Coxopleurae produced beyond the last ventral plate two-thirds to

three-fourths the length of the latter; process terminating in 3 points or spines and with a lateral spine on the ectal margin.

Between 7 and 12 first pairs of legs with the tarsi bearing 2 spines, the others with a single tarsal spine, excepting the penult and anal pairs which have none.

Prefemur of anal legs with a row of 3 strictly ventral spines; along mesoventral edge with a series of 3 spines; and along mesodorsal edge with a third series also of 3 spines, the distal one of the last mentioned being at the corner; the median spine in each series largest. Other joints wholly smooth.

Length, 50 to 55 mm.

Locality.—Not definitely known; but probably India. It is certainly from the Eastern Hemisphere, as shown by the presence of spines on the anal legs. The type specimens are in the Mus. of Comp. Zoology at Cambridge.

Bulletin of the Museum of Comparative Zoölogy

AT HARVARD COLLEGE.

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THE LITHOBIID GENERA NAMPABIUS, GARIBIUS, TIDABIUS, AND SIGIBIUS.

BY RALPH V. CHAMBERLIN.

WITH FIVE PLATES.

**CAMBRIDGE, MASS., U. S. A.,
PRINTED FOR THE MUSEUM.**

NOVEMBER, 1913.

No. 2.— *The Lithobiid Genera Nampabius, Garibius, Tidabius, and Sigibius.*

BY RALPH V. CHAMBERLIN.

THE four new genera dealt with in the present paper include the smallest of the known North American species of Lithobiidae. None of them exceeds ten mm. in length, most of them averaging materially less than this. They occur principally among the layers of damp fallen leaves, under the bark of logs, and in the looser loam of open woods. All seem to have the death-feigning instinct more or less pronounced, when suddenly jarred or otherwise frightened often flexing the body laterally into a circle and lying rigid for some time, when they may be handled with forceps or otherwise without their relaxing. Because of their small size and the effectiveness with which they conceal themselves, specimens of these species are not often secured by the general collector; but one looking for them especially and understanding their habits may nearly always secure one or more species in appropriate habitats throughout the United States excepting the Southwest where they are replaced by *Oabius* (*pylorus* Chamb., *tiganus* Chamb., etc.), *Hesperobius* (*medius* Chamb., *obesus* Stuxb., and allies), and related genera to be considered in a succeeding Bulletin. They may sometimes be secured in considerable numbers by sifting leaves.

In addition to the similarity in many features of habit, the species of these four genera likewise have much in common in general appearance and structure. The genera are, however, readily separated upon critical examination. The discrimination of the species has presented more difficulty. Heretofore the species have remained practically unknown, not only because of the paucity of material in collections, but especially because the characters studied and given in descriptions are, in the main, not truly specific, being either generic in extent or common to a number of species. More detailed and careful study and comparison of the material in extensive collections made by the author personally in various parts of the country has revealed the considerable number of species here described.

The four genera may readily be separated by means of the following key.

Key to the Genera.

- a. Articles of antennae from 25 to 35, the number being mostly near 32. Posterior legs of ♂ without special processes or lobes. (Posterior coxae wholly unarmed).
 - b. Dorsal spines of anal and penult legs 0, 0, 1, 0, 0, ventral 0, 1, 1, 1, 0. *Sigibius*, gen. nov.
 - bb. Dorsal spines of anal legs 0, 0, 2, 0, 0, ventral 0, 1, 3, 1, 0, or 0, 1, 3, 2, 0; dorsal spines of penult legs 0, 0, 3, 1, 0, or 0, 0, 3, 1, 1, ventral 0, 1, 3, 3, 1 or 0, 1, 2, 3, 1. *Tidabius*, gen. nov.
- aa. Articles of antennae normally 20. Either anal or penult legs of ♂ with a special lobè. (Coxae armed or unarmed).
 - b. Posterior coxae wholly unarmed; median incision of prosternum semicircular; penult legs of ♂ always bearing at distal end of tibia on dorsal side a small but conspicuous lobe, the anal legs without lobes or crests; posterior angles of 11th and 13th or at least of 13th more or less produced. *Nampabius*, gen. nov.
 - bb. Last 3 or 4 pairs of coxae dorsally armed; median incision of prosternum V-shaped; penult legs of ♂ without any such lobe, but anal legs bearing at distal end of tibia a small crest; posterior angles of none of the dorsal plates at all produced. *Garibius*, gen. nov.

NAMPABIUS, gen. nov.

Body conspicuously attenuated cephalad, with the first dorsal plate much narrower than the head and also narrower than the third plate. Mostly robust, varying from 6 to 8.5 times as long as the width of the 10th plate.

Antennae short; composed of 20 articles.

Ocelli in 2, or, less commonly, in 3, longitudinal series; the single ocellus mostly a little smaller than the most caudal one of the dorsal series.

Prosternal teeth 2+2. Median incision semicircular in outline, never acute at bottom. (Plate 1, fig. 2).

Eleventh and thirteenth dorsal plates with the caudal angles more or less produced or sometimes that of thirteenth alone distinctly extended; angles of other plates straight or excised.

Claw of the female gonopods tripartite, with the lobes subequal or

the median longest, all usually more or less rounded distally. Basal spines 2+2, long and usually slender, the sides, as seen in ventral view, converging to near the apex or beginning of the acuminate distal portion which is very short. (Plate 2, fig. 6).

Coxal pores very small; circular, 2, 2, 2, 2 to 2, 3, 3, 3 in number.

Ventral spines of the anal legs 0, 1, 1, 0, 0 or 0, 1, 1, 1, 0; dorsal 0, 0, 0, 0, 0 or 0, 0, 2, 0, 0; claws 2 or, rarely, but 1. Ventral spines of penult legs 0, 1, 1, 1, 0 or 0, 1, 2, 1, 0; dorsal spines 0, 0, 0, 0, 0 or 0, 0, 2, 0, 0; claws 2 or, rarely, but 1. Ventral spines of thirteenth legs 0, 0, 2, 1, 1 to 0, 0, 0, 1, 1; dorsal from 0, 0, 0, 0, 0 to 0, 0, 2, 1, 1. Ventral spines of twelfth legs from 0, 0, 0, 0, 1 to 0, 0, 0, 2, 1; dorsal from 0, 0, 0, 0, 0 to 0, 0, 2, 1, 2. Ventral spines of eleventh legs 0, 0, 0, 0, 1 to 0, 0, 0, 2, 1; dorsal from 0, 0, 0, 0, 1 to 0, 0, 0, 2, 2. First two to first four pairs of legs with no ventral spines on any joint; and the third joint of the first twelve or thirteen pairs of legs ventrally unarmed. The posterior coxae are wholly unarmed.

Anal legs in the male short; moderately and uniformly inflated; without special lobes or furrows. Penult legs with the fifth article produced at its distal end on the dorsal or dorsomesal side into a small sub-cylindric or distally expanded lobe bearing a number of sensory hairs.

TYPE.—*N. virginensis*, sp. nov.

DISTRIBUTION.—Eastern and Southeastern States, in the latter section occurring chiefly in or near the mountainous parts.

The species of this genus are the smallest members of the family, all known forms being under 7.5 mm. in length. In proportions they are mostly relatively robust and conspicuously narrowed cephalad. They vary considerably in color, some being testaceous, some ferruginous, and others chestnut. While the head may be paler than the dorsum, it is usually darker, frequently being a shining reddish black. As in *Tidabius*, the posterior legs, especially distally, are nearly always more brightly pigmented, being commonly bright yellow or orange. These forms occur commonly in and beneath layers of fallen leaves and loose piles of sticks.

The following key will aid in the identification of the known species.

Key to species of Nampabius.

- a. Neither the anal nor the penult legs with any dorsal spines.
Ventral spines of anal legs 0, 1, 1, 0, 0.
N. fungiferopes (Chamberlin).
- aa. Anal and penult legs with the third joint armed with 2 dorsal spines.

- b. Dorsal spines of twelfth legs 0, 0, 2, 1, 2; (ventral spines of ninth to twelfth legs 0, 0, 0, 2, 1). *N. virginiensis*, sp. nov.
- bb. Dorsal spines of twelfth legs not 0, 0, 2, 1, 2.
- c. Third joint of twelfth legs unarmed dorsally (spines 0, 0, 0, 1, 2 or fewer).
- d. Dorsal spines of twelfth legs 0, 0, 0, 0 (1), 1; of the eleventh 0, 0, 0, 1, 1. *N. parienus*, sp. nov.
- dd. Dorsal spines of twelfth legs 0, 0, 0, 1, 2; of the eleventh 0, 0, 0, 1, 2 or 0, 0, 0, 2, 2.
- e. Dorsal spines of tenth and eleventh legs 0, 0, 0, 1, 2; ventral spines of thirteenth legs 0, 0, 1, 1, 1. *N. embius*, sp. nov.
- ee. Dorsal spines of eleventh legs 0, 0, 0, 2, 2; ventral spines of thirteenth legs 0, 0, 2, 1, 1.
- f. Ventral spines of twelfth legs 0, 0, 1, 2, 1; of eighth to eleventh 0, 0, 0, 2, 1; ocelli cir. 6 in two series. *N. inimicus*, sp. nov.
- ff. Ventral spines of twelfth legs 0, 0, 0, 1, 1; of eighth to eleventh also 0, 0, 0, 1, 1; ocelli 10 or more in three series. *N. georgianus*, sp. nov.
- cc. Third joint of twelfth legs armed with one or two dorsal spines.
- d. Dorsal spines of twelfth legs 0, 0, 1, 1, 2 or 0, 0, 2, 1, 1; of tenth and eleventh 0, 0, 0, 2, 2. *N. carolinensis*, sp. nov.
- dd. Dorsal spines of twelfth legs 0, 0, 2, 1, 0 or 0, 0, 2, 1, 1; of tenth and eleventh 0, 0, 0, 0, 1 or 0, 0, 0, 1, 1.
- e. Dorsal spines of twelfth and thirteenth legs 0, 0, 2, 1, 0; of eleventh 0, 0, 0, 1, 1. *N. tennesseensis*, sp. nov.
- ee. Dorsal spines of twelfth and thirteenth legs 0, 0, 2, 1, 1; of eleventh 0, 0, 0, 0, 1.
- f. Near 8.5 times longer than width of tenth plate. *N. longiceps*, sp. nov.
- ff. Not more than 7 times longer than width of tenth plate. *N. lulae*, sp. nov.

NOTE.—The key does not include *N. lundii* (Meinert) the insufficient description of which must leave its identity in doubt until the types can be restudied. See further under the account of this species.

NAMPABIUS FUNGIFEROPES (Chamberlin).

Lithobius fungiferopes Chamberlin, Proc. Acad. nat. sci. Phil.,
1904, 56, p. 652.

DIAGNOSIS.—Dorsum testaceous to dark, more or less reddish brown, in life more or less suffused with purplish. Head darker, almost black, in front of suture paler and with a tendency toward ferruginous.

Body about $6\frac{1}{2}$ times longer than width of tenth plate.

Head considerably wider than long.

Ocelli mostly 4 to 6 in two series.

Prosternum about 1.54–1.6 times wider than long; distance between chitinous spots about 2.3–2.4 times greater than width at median incision and 3.8 or 3.9 times the dental line.

First dorsal plate about 1.9 times wider than long.

Coxal pores 2, 2, 2, 2.

Claw of female gonopods short and wide with the median lobe considerably largest, the lateral equal. Spines of moderate width, the sides converging distad to the short, abruptly subacute apex.

Twelfth to anal legs inclusive with no dorsal spines. Ventral spines of anal 0, 1, 1, 0, 0; of penult, 0, 1, 1, 1, 0. Ventral spines of ninth to thirteenth legs (also of fifth to ninth) inclusive 0, 0, 0, 0, 1. Dorsal spines of tenth and eleventh legs (also of second to tenth) 0, 0, 0, 0, 1.

Process of the penult legs in the male conspicuously expanding distad; bearing the usual hairs. Tarsal joints abruptly and considerably more slender than the tibia. (Plate 1, fig. 6–7).

DESCRIPTION.—Above testaceous to dark, more or less reddish, brown, in life especially commonly conspicuously suffused with violaceous or purplish. Head deeper in color, almost black, but paler in front of suture where it may show a tendency toward ferruginous. Antennae rufous distally, proximally brown or purplish brown. Prosternum dark brown with the prehensorial feet lighter. Venter light brown or testaceous, in life suffused with purplish. Legs pale brown, in life of a distinctly purplish or violaceous tinge proximally, being yellow distally; posterior pairs more strongly pigmented as usual, bright yellow except proximally.

Body strongly attenuated cephalad of tenth plate as usual. Proportionately robust, being mostly 6.75+ times longer than width of tenth plate. The widths of head and first, third, eighth, tenth, and twelfth plates to each other as 37:31:33:42:42:39.

Head wider than long (mostly from 37:34 to 37:36); widely rounded anteriorly. Hairs long and sparse.

Antennae short; composed of the normal twenty articles, all of which are short. Hairs of moderate length, not very dense, more sparse proximally.

Eyes composed mostly of from 4 to 6 ocelli which are arranged in two series:—thus, 1+2, 1 to 1+3, 2. The single ocellus smaller than most of the others. All ocelli well pigmented.

Prosternum 1.54–1.6 times wider than long. Distance between chitinous spots near 2.38 times the width at level of bottom of mesal incision; about 3.87 times the dental line, the anterior portion being more strongly narrowed than in the related species. Median incision shallow, not large, of the usual semicircular form. Teeth small and acute, equal or nearly so, the interval between each pair narrow and well rounded at bottom. Sides slanting directly from close to base of each ectal tooth, nearly straight, being slightly concave near middle of length.

First dorsal plate widest at anterior end, the sides at first but little and then more strongly converging caudad and rounding in about the caudal corners; nearly 1.9 times wider than long. Posterior corners of the eleventh and more distinctly of the thirteenth plates weakly produced, those of the ninth straight.

Coxal pores small and circular; mostly 2, 2, 2, 2.

Spines of first legs $\frac{0, 0, 0, 0, 0}{0, 0, 0, 0, 0}$; of second to fourth inclusive $\frac{0, 0, 0, 0, 1}{0, 0, 0, 0, 1}$; of fifth to eleventh inclusive $\frac{0, 0, 0, 0, 1}{0, 0, 0, 0, 1}$; of twelfth and thirteenth, $\frac{0, 0, 0, 0, 0}{0, 0, 0, 0, 1}$; of the penult $\frac{0, 0, 0, 0, 0}{0, 1, 1, 1, 0}$, the claws 2; of the anal $\frac{0, 0, 0, 0, 0}{0, 1, 1, 0, 0}$ claws 2, the accessory claw easily lost from preserved specimens in both anal and penult pairs.

In the male the anal and penult legs are short and but moderately inflated; longitudinally sulcate along ectal or anterior side, more distinctly so on fourth and fifth articles. In the penult legs the tarsal joints are abruptly and considerably more slender than the tibia; tibia with the distal mesodorsal corner obliquely complanate and there bearing the usual small process, this being in this species conspicuously widened at the distal end (see Plate 1, fig. 6 and 7) and bearing on its distal surface the usual sensory hairs.

Claw of female gonopods short and relatively wide, scarcely curved; tripartite, the middle lobe considerably larger than the two lateral which are about equal, all lobes distally narrowly rounded. Spines

moderately wide, with the sides converging from base distad, toward ends more abruptly bending in to the acute or subacute apex. Mesal side of first article of gonopods nearly straight and parallel with its fellow.

Length from 5 to nearly 7 mm.

TYPE LOCALITY.—New York (Ithaca!).

LOCALITIES.—New York (Ithaca!); Vermont (Jamaica! Grout's Mill!).

NAMPABIUS VIRGINIENSIS, sp. nov.

DIAGNOSIS.—Dorsum testaceous to dark reddish or ferruginous brown. Head more or less ferruginous, darker caudad of suture.

Body robust, being mostly but 6.25 or 6.3 times longer than width of the tenth plate.

Head equal in length and breadth; cordate.

Ocelli mostly 6 or 7 in two series.

Prosternum about 1.63 times wider than long, the anterior portion being short. Distance between chitinous spots only 1.7 times greater than width at median incision and but 2.9 times greater than the dental line.

First dorsal plate nearly 1.9 times wider than long.

Coxal pores 2, 2, 2, 2-2, 3, 3, 3.

Claw of female gonopods with the outer latest tooth somewhat smaller than the inner. Spines long, their sides moderately converging to near apex where curving in more abruptly to the acute apex.

Dorsal spines of anal and of penult legs 0, 0, 2, 0, 0; ventral spines of these legs 0, 1, 1, 1, 0. Dorsal spines of thirteenth legs 0, 0, 2, 1, 0; ventral 0, 0, 2, 1, 1. Dorsal spines of twelfth legs 0, 0, 2, 1, 2. Dorsal spines of tenth and eleventh legs 0, 0, 0, 2, 2. Ventral spines of ninth to twelfth legs inclusive 0, 0, 0, 2, 1.

Process of penult legs of male subcylindric; expanded somewhat distally, flat-topped and on the distal surface bearing a subdense patch of moderately long sensory hairs. (Plate 1, fig. 4-5).

DESCRIPTION.—Dorsally testaceous to dark reddish or ferruginous brown, more reddish anteriorly and posteriorly. Head more or less ferruginous, darker, dusky, caudad of the suture. Antennae like the head; pale at tips. Prosternum brown, with the prehensorial feet paler. Venter pale yellowish to light reddish brown, the caudal plates darker. Legs pale yellow to testaceous, with the posterior pairs, excepting at base, bright yellow or somewhat orange.

Body attenuated as usual. Typically near 6.25 or 6.3 times longer than width of the tenth plate. A female 5 mm. long has the widths of head and first, third, eighth, tenth, and twelfth plates to each other as 37:33:35:42:42:42.

Head nearly equal in length and breadth. Cordate; widest a little in front of marginal breaks from where it narrows continuously forwards.

Antennae very short; moderately attenuated. Composed of the usual twenty articles which are short and very short; decreasing distad from the second.

Eyes composed mostly of 6 or 7 ocelli arranged in 2 series:—thus 1+3, 2 or 1+4, 2. Dark. The most caudal ocellus of upper row considerably larger than the single one, the first of lower row less markedly larger.

Prosternum about 1.63 times wider than long. Distance between chitinous spots only 1.7 times greater than width at level of bottom of mesal incision which is nearly in line with caudal ends of lateral slopes; 2.9 times longer than the dental line which is also thus longer than usual. Median incision large and conspicuous, of the usual form. Teeth subequal, small and acute, the interval between each pair semicircular; line of apices recurved. Sides straight or a little incurved, short.

First dorsal plate widest at anterior end from where the sides converge caudad considerably; nearly 1.9 times wider than long. Thirteenth dorsal plate with posterior corners moderately produced, those of the eleventh less so as usual; caudal margin of ninth plate straight.

Coxal pores small; 2, 2, 2, 2 to 2, 3, 3, 3.

Spines of the first legs $\frac{0, 0, 0, 0, 0, 1}{0, 0, 0, 0, 1}$; of the second $\frac{0, 0, 0, 0, 1}{0, 0, 0, 0, 1}$; of the third to seventh inclusive, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 0, 1}$; of the eighth $\frac{0, 0, 0, 1, 2}{0, 0, 0, 1, 1}$; of the ninth, $\frac{0, 0, 0, 1, 2}{0, 0, 0, 2, 1}$; of the tenth and eleventh, $\frac{0, 0, 0, 2, 2}{0, 0, 0, 2, 1}$; of the thirteenth $\frac{0, 0, 2, 1, 0}{0, 0, 2, 1, 1}$; of the penult $\frac{0, 0, 2, 0, 0}{0, 1, 1, 1, 0}$; of the anal $\frac{0, 0, 2, 0, 0}{0, 1, 1, 1, 0}$.

In the male the anal legs are short, moderately and uniformly inflated, and without special modifications. Penult legs similar to anal but the fifth article obliquely complanate at distal end on meso-dorsal side and there bearing the usual small lobe which is subcylindric and somewhat expanded distad, especially on side toward distal end of leg; sensory hairs borne on distal surface of lobe rather long and subdense. (Plate 1, fig. 4-5).

Claw of female gonopods of moderate size; considerably curved; tripartite; the inner and median teeth not much different in size, the outer one smaller. Spines rather long, the sides as usual converging from base distad, toward end more strongly curving in to meet at the acute apex. Inner side of first article nearly straight, a little diverging from its fellow proximad. Length near 5 mm.

TYPE LOCALITY.— Virginia (Natural Bridge! August, 1910).

LOCALITIES.— Virginia (Natural Bridge!); Tennessee (Unaka Springs!).

NAMPABIUS PARIENUS, sp. nov.

DIAGNOSIS.— Dorsal spines of anal, penult, and thirteenth legs 0, 0, 2, 0, 0. Ventral spines of anal leg 0, 1, 1, 1, 0, of penult 0, 1, 1, 1, 0–0, 1, 2, 1, 0; of ninth to thirteenth, 0, 0, 0, 1, 1. Dorsal spines of tenth and eleventh pairs of legs (as also of the second or third to ninth) 0, 0, 0, 1, 1.

Ocelli 5 or 6 in two series.

Prosternum 1.57 or 1.6 times wider than long. Distance between chitinous spots about 3.6 times greater than the distal line; 2.25 times longer than the width at median incision.

Coxal pores 2, 2, 2, 2–2, 3, 3, 2.

Process of tibia of penult legs in male rather long and slender, in proximal aspect with lateral lines parallel; in lateral aspect appearing thicker and projecting over base on side at distal end of article; sensory hairs long and slender.

Body 7.7 or 7.75 times longer than width of tenth plate.

Head broadly subcordate; distinctly wider than long.

First dorsal plate (in type) between 1.75 and 1.8 times wider than long.

Dorsum yellow with the caudal plates tinged with orange. Head orange, with the frontal region paler.

DESCRIPTION.— The body above is yellow with the caudal plates tinged with orange. Head orange, the frontal region paler, more yellowish. Antennae orange, yellow at tips. Prosternum orange brown. Venter pale, the caudal plates more strongly pigmented as usual. Legs pale excepting the posterior pairs which are bright orange.

Body more slender than in most related species, in the type being 7.73 times longer than the width of the tenth dorsal plate. Width of head and first, third, eighth, tenth, and twelfth plates to each other about as 36:32:35:43:44:41.

Head broadly subcordate, considerably bulging caudad of eyes. Clearly wider than long (12: 11). Hairs short and sparse.

Antennae short. Articles short and very short, decreasing distad of the second as usual. Hairs short and not dense.

Eyes typically composed of five or six ocelli in two series:—thus, 1+2, 2 or 1+3, 2. Single ocellus smaller than the first one of upper row and the others of the usual relative sizes.

Prosternum in type 1.57 times wider than long. Distance between chitinous spots 3.6 times the dental line; 2.25 times width at level of bottom of median incision. Median incision of the usual semicircular form, rather small, the distance across it between the two mesal teeth not greater than that between the teeth of each pair. Teeth moderate; line of apices distinctly and considerably recurved. Spine at base of ectal tooth; slender, somewhat curved mesad distally, not exceeding the tooth in length.

First dorsal plate widest near anterior end, the sides from there at first slightly and then more strongly converging caudad; plate in type 1.77+ times wider than long. Posterior corners of the eleventh and thirteenth dorsal plates a little produced, those of the ninth straight or also showing a very slight tendency toward production, rounded; other minor plates with corners oblique or rounded.

Coxal pores very small; 2, 2, 2, 2-2, 3, 3, 2.

Spines of first legs, $\frac{0, 0, 0, 0, 1}{0, 0, 0, 0, 0}$; of second, $\frac{0, 0, 0, 0, (1), 1}{0, 0, 0, 0, 0}$; of third, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 0, 0}$; of the fourth to seventh, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 0, 1}$; of the ninth to eleventh, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 1, 1}$; of the twelfth, $\frac{0, 0, 0, 0, (1), 1}{0, 0, 0, 1, 1}$, the dorsal spine of the fifth article being on the cephalic side; of the thirteenth, $\frac{0, 0, 2, 0, 0}{0, 0, 0, 1, 1}$; of the penult, $\frac{0, 0, 2, 0, 0}{0, 1, 1, 1, 0-0, 1, 2, 1, 0}$, claws 2; of the anal, $\frac{0, 0, 2, 0, 0}{0, 1, 1, 1, 0}$, claws 2.

In the male the anal and penult legs are short and uniformly and but little inflated; both are laterally longitudinally sulcate. The process at the distal end of the tibia of the penult legs is rather long and slender; when viewed from proximal aspect of leg its sides appear nearly parallel; in lateral aspect the process is somewhat thicker and projects distad above its base; distal surface rising obliquely dorso-distad from its proximal end to its distal; sensory hairs long and slender. (Plate 3, fig. 1-2).

Length (type) 6.5 mm.; width of tenth plate .84 mm.; length of antennae 2 mm.

LOCALITY.—North Carolina (Hot Springs!).

NAMPABIUS EMBIUS, sp. nov.

DIAGNOSIS.—Dorsal spines of the anal and penult legs 0, 0, 2, 0, 0. Dorsal spines of the thirteenth legs 0, 0, 2, 1, 0; ventral 0, 0, 1, 1, 1. Dorsal spines of the tenth to twelfth legs 0, 0, 0, 1, 2; ventral 0, 0, 0, 2, 1. Dorsal spines of the sixth to ninth legs also 0, 0, 0, 1, 2.

Ocelli 6 to 8 in two series.

Prosternum about 1.56 times wider than long. Distance between chitinous spots 2.14 times width at median incision; $3\frac{1}{2}$ times the dental line.

Spines of the gonopods rather long; sides parallel or but slightly converging from base to the very short acuminate tip, the sides of which are straight and meet at an almost obtuse angle.

Body more slender than in most related species being (in type) about $8\frac{1}{2}$ times longer than the width of the tenth dorsal plate.

Head clearly wider than long.

First dorsal plate near 1.75 times wider than long.

Dorsum brown of dilute chestnut caste. Head dark brownish red.

DESCRIPTION.—Brown of dilute chestnut caste. Head dark brownish red. Antennae brown, pale distally. Prosternum dark, more brownish than the head with the prehensorial feet paler. Venter pale brown, the caudal plates darker. Legs colored nearly like the corresponding plates of venter; posterior pairs light distad.

Body comparatively slender being in the type $8\frac{1}{2}$ times longer than the width of the tenth plate. Widths of head and of the first, third, eighth, tenth, and twelfth plates to each other about as 39:35:38:48:49:47.5.

Head wider than long in ratio 13:12; widest behind eyes from where the convergence of the sides to the caudal corners is marked.

Antennae short as usual. Moderately attenuated. The twenty articles short and very short, decreasing regularly distad. Hairs of moderate length, short, not dense. (Plate 2, fig. 7).

Eyes composed of 6 to 8 ocelli in two series:—thus, 1+3, 3—1+4, 3—1+3, 2. Ocelli of moderate size, black.

Prosternum 1.56 times wider than long. Distance between chitinous spots 2.14 times width at level of bottom of mesal incision; $3\frac{1}{2}$ times the dental line. Mesal incision wide and shallow, of the usual form at bottom. Teeth small, distally narrowly rounded; line of apices rather strongly recurved. Spine unusually long, bristle like.

First dorsal plate widest a little caudad of anterior end from where

the sides converge considerably; 1.75 times wider than long. Posterior corners of the thirteenth plate a little produced; those of the eleventh nearly straight and those of ninth rounded cephalad.

Coxal pores small and circular as usual, 2, 3, 3, 3.

Spines of first pair of legs, $\frac{0, 0, 0, 0, 0}{0, 0, 0, 0, 0}$ to $\frac{0, 0, 0, 0, 1}{0, 0, 0, 0, 0}$; of second and third, $\frac{0, 0, 0, 0, 1}{0, 0, 0, 0, 0}$; of fourth and fifth, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 0, 1}$; of sixth, $\frac{0, 0, 0, 1, 2}{0, 0, 0, 0, 1}$ or $\frac{0, 0, 0, 1, 2}{0, 0, 0, 0, 1}$; of seventh, $\frac{0, 0, 0, 1, 2}{0, 0, 0, 1, 1}$; of tenth to twelfth, $\frac{0, 0, 0, 1, 2}{0, 0, 0, 2, 1}$; of thirteenth, $\frac{0, 0, 2, 1, 0}{0, 0, 1, 1, 1}$; of the penult, $\frac{0, 0, 2, 0, 0}{0, 1, 1, 1, 0}$; claws 2; of the anal 0, 0, 2, 0, 0.

Claw of female gonopods of moderate size; distinctly tripartite. Spines rather long, with sides parallel or but slightly converging from base to acuminate apical portion which, as usual, is relatively very short with sides strictly straight and running to an almost obtuse angle. Inner side of first article of gonopod considerably diverging proximad from its fellow. Sternite mesally straight, with no distinct process.

Length (type) 7.5 mm., width of tenth plate .91 mm.; length of antenna, 2 mm.

LOCALITY.—South Carolina (Taylors!).

NAMPABIUS INIMICUS, sp. nov.

DIAGNOSIS.—Dorsal spines of penult legs 0, 0, 2, 0, 0; ventral 0, 1, 2, 1, 0. Dorsal spines of thirteenth legs, 0, 0, 2, 1, 0; ventral, 0, 0, 2, 1, 1. Dorsal spines of twelfth legs, 0, 0, 0, 1, 2; ventral, 0, 0, 1, 2, 1. Dorsal spines of tenth and eleventh legs, 0, 0, 0, 2, 2. Ventral spines of tenth and eleventh (as also of eighth and ninth) legs, 0, 0, 0, 2, 1.

Ocelli (in type) 6 in two series.

Prosternum about 1.57 times wider than long. Distance between chitinous spots 2.1 times the width at median incision; 3.1 times as great as the dental line.

Coxal pores 2, 2, 2, 2-3, 3, 2.

Body about 7.8 times longer than width of tenth dorsal plate.

Head subrotund, considerably wider than long.

First dorsal plate 1.7 times wider than long.

Process of penult legs in male small, cylindric proximally but expanded at distal end on side toward distal end of joint; sensory hairs short and spinescent. (Plate 3, fig. 5-6).

Dorsum light testaceous, the caudal plates of ferruginous tinge. Head dark orange.

DESCRIPTION.—Dorsum light testaceous, the caudal plates of ferruginous or orange tinge. Head dark orange. Antennae reddish brown, light at tips. Prosternum brown. Venter very pale, excepting the caudal plates which are orange. Legs pale like the venter, but the posterior pairs more brightly pigmented as usual.

Body more slender than typical in the genus the length of type specimen being 7.77+ times greater than the width of the tenth plate. Width of head and of first, third, eighth, tenth, and twelfth dorsal plates to each other as 39:34:37:46:47:43 (σ^7).

Head subrotund; considerably wider than long (39:35); the sides convexly bulging caudad of eyes; widely rounded cephalad.

Antennae moderately short. Joints short, decreasing markedly in size from the second distad. Sides of articles straight, moderately flaring distad. Hairs moderately short, not very dense.

Eyes in type composed of 6 ocelli in two series:—thus, 1+3, 2. Single ocellus of about same size as the first one of upper series or a very little smaller; others decreasing in size cephalad.

Prosternum 1.57+ times wider than long. Distance between chitinous spots 2.1 times width at level of bottom of median incision; 3.1 times the dental line. Lateral edges nearly straight or very slightly convex cephalad and slightly bending ectad at posterior end. Median incision wide, semicircular as usual; the interval between the two mesal teeth decidedly greater than that between the teeth of each lateral pair. Teeth acute; line of apices distinctly recurved.

First dorsal plate 1.7 times wider than long; widest near middle, the sides converging cephalad and, more conspicuously, caudad. Posterior angles of eleventh and thirteenth dorsal plates a little produced as usual, those of the other minor plates oblique or simply rounded.

Coxal pores very small and circular; 2, 2, 2, 2—2, 3, 3, 2.

Spines of first legs, $\frac{0}{0}, \frac{0}{0}, \frac{0}{0}, \frac{0}{0}, \frac{1}{0}$; of the second, $\frac{0}{0}, \frac{0}{0}, \frac{0}{0}, \frac{1}{0}, \frac{1}{0}$ or $\frac{0}{0}, \frac{0}{0}, \frac{0}{0}, \frac{0}{0}, \frac{1}{0}$; ventral spines of fourth and fifth, 0, 0, 0, 0, 1 and of the sixth the same or else 0, 0, 0, 1, 1; spines of seventh legs, $\frac{0}{0}, \frac{0}{0}, \frac{0}{0}, \frac{1}{0}, \frac{2}{1}$; of eighth and ninth, $\frac{0}{0}, \frac{0}{0}, \frac{0}{0}, \frac{1}{0}, \frac{2}{1}$ of tenth and eleventh, $\frac{0}{0}, \frac{0}{0}, \frac{0}{0}, \frac{2}{1}, \frac{2}{1}$; of twelfth, $\frac{0}{0}, \frac{0}{0}, \frac{1}{0}, \frac{2}{1}$; of thirteenth, $\frac{0}{0}, \frac{0}{0}, \frac{2}{1}, \frac{0}{1}$; of the penult, $\frac{0}{0}, \frac{0}{1}, \frac{2}{1}, \frac{0}{0}$; anal lost.

Penult legs of male short and uniformly inflated. Process at distal

end of tibia small, cylindric at base and expanded at free end on side toward distal end of joint; process extending dorsodistad or also a little caudad; sensory hairs on distal surface of process short and spinescent. (Plate 3, fig. 5-6).

Length of type 7 mm.; width of tenth plate .9 mm.; length of antenna, 2.3 mm.

LOCALITY.—Tennessee (Russellville!).

NAMPABIUS GEORGIANUS, sp. nov.

DIAGNOSIS.—Dorsal spines of anal and penult legs 0, 0, 2, 0, 0; ventral 0, 1, 1, 1, 0. Dorsal spines of thirteenth legs 0, 0, 2, 1, 0; ventral 0, 0, 2, 1, 1. Dorsal spines of twelfth legs, 0, 0, 1, 2; ventral, 0, 0, 0, 1, 1. Dorsal spines of eleventh legs, 0, 0, 0, 2, 2; of the tenth, 0, 0, 0, 1, 2. Ventral spines of tenth and eleventh legs (as also of the sixth to ninth) 0, 0, 0, 1, 1.

Ocelli 10-12 in three series.

Prosternum 1.5 times wider than long; distance between chitinous spots only 3.1 times as great as dental line; about $2\frac{1}{3}$ times as great as width at median incision.

Body 6.6 to nearly 7 times longer than width of tenth dorsal plate.

Head much wider than long.

First dorsal plate twice as wide as long or nearly so.

Process of tibia of penult leg of male small and subcylindric, (Plate 2, fig. 2).

Dorsum testaceous. Head light ferruginous; frontal region palest.

DESCRIPTION.—Dorsum testaceous. Head somewhat light ferruginous with caudal portion covered with a darker brown area, leaving the frontal region palest. Antennae somewhat pale ferruginous brown, lighter at tips. Prosternum brown of more or less reddish caste, the prehensorial feet paler. Venter pale yellow, with the caudal plates darker. Legs like adjoining part of venter with caudal pairs somewhat brighter, distally.

Body strongly narrowed cephalad, moderately robust, being from 6.6 to 6.9 times longer than width of the tenth plate, being thus relatively more slender than in *N. virginensis*. Widths of head and of first, third, eighth, tenth, and twelfth plates as 37:34:38:46:48:43.

Head much wider than long (average ratio about 33.5:29.5), being wider by from $\frac{1}{8}$ th to $\frac{1}{10}$ th. Broadest a little back of eyes where it bulges considerably on each side. Broad anteriorly, not subcordate

as in *virginiensis*. Hairs moderately short, very sparse. (Plate 3, fig. 8).

Antennae short and but moderately attenuated. Articles, as usual, 20, which are short though rather longer than in *virginiensis* and also less closely telescoped; decreasing distad from the second. Hairs moderately short, not especially dense.

Eyes composed of ten to twelve ocelli arranged in three series:—thus 1+4, 4, 3-1+4, 3, 3. Ocelli black; closely but somewhat irregularly arranged.

Prosternum 1.5 times wider than long. Distance between chitinous spots 3.1 times as great as the length of dental line; about $2\frac{1}{2}$ times the width at level of bottom of median incision which is clearly farther cephalad of caudal ends of lateral slopes than in *virginiensis*. Sides or slopes of anterior portion straight, slanting directly ecto-caudad from the teeth. Teeth small and acute. Median incision conspicuous, of the usual semicircular form.

First dorsal plate widest at anterior end, the sides at first moderately converging and then strongly rounding in about the caudal corners; about twice as wide as long. Ninth plate with caudal margin bent forward a little at ends. Eleventh plate with caudal margin straight all the way across or very nearly so. Thirteenth plate with caudal corners a little produced.

Coxal pores small; 2, 2, 2, 2.

Spines of first legs, $\frac{0, 0, 0, 0, 0}{0, 0, 0, 0, 0}$; of second, $\frac{0, 0, 0, 0, 1}{0, 0, 0, 0, 0}$, 1; of third, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 0, 0}$; of fourth and fifth, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 0, 1}$; of sixth, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 1, 1}$; of eighth to tenth inclusive, $\frac{0, 0, 0, 1, 2}{0, 0, 0, 1, 1}$; of eleventh, $\frac{0, 0, 0, 2, 2}{0, 0, 0, 1, 1}$; of twelfth, $\frac{0, 0, 0, 1, 2}{0, 0, 0, 1, 1}$; of thirteenth, $\frac{0, 0, 2, 1, 0}{0, 0, 2, 1, 1}$; of penult and anal, $\frac{0, 0, 2, 0, 0}{0, 1, 1, 1, 0}$, each with two claws.

In the male the anal legs are short; uniformly and but little crassate. (Plate 2, fig 3). Penult legs similar to anal but bearing the characteristic lobe at distal end of the fifth joint, or tibia; lobe small and subcylindric, proportionately longer than in *virginiensis* (Plate 2, fig. 1-2); extending in a mesodorsal and somewhat distal direction, bearing on the free surface a limited number of rather short sensory hairs.

Length 5 to 6.25 mm.

LOCALITY.—Georgia (Tallulah Falls!).

NAMPABIUS CAROLINENSIS, sp. nov.

DIAGNOSIS.— Dorsal spines of the anal and penult legs, 0, 0, 2, 0, 0; ventral of penult, 0, 1, 2, 1, 0, of anal 0, 1, 1, 1, 0. Dorsal spines of thirteenth legs, 0, 0, 2, 1, 0; ventral, 0, 0, 2, 1, 1. Dorsal spines twelfth legs, 0, 0, 1, 1, 2. Dorsal spines of tenth and eleventh legs, 0, 0, 0, 2, 2. Ventral spines of ninth to twelfth (as also of seventh and eighth) legs, 0, 0, 0, 1, 1.

Ocelli near 6 in number, in two series.

Prosternum 1.6 times wider than long. Distance between chitinous spots only 2.1 times width at median incision; 3.5 times as great as dental line.

Body near eight times longer than width of tenth plate.

Head conspicuously wider than long.

First dorsal plate near 1.7+ times wider than long.

Process of tibia of penult leg in male relatively slender and moderately long, broadening at base but not distally where it is rounded. Hairs at distal end straight and divergent. (Plate 2, figs. 4-5).

Dorsum yellowish, uniform. Head reddish orange.

DESCRIPTION.— Dorsum yellowish, uniform. Head reddish orange. Antennae brown, pale distally. Prosternum somewhat paler than head, the prehensorial feet still lighter. Venter light yellow, the caudal plates orange. Legs nearly like corresponding plates of venter.

Body moderate, being 7.9 or 8 times longer than width of tenth plate. Widths of head and of first, third, eighth, tenth, and twelfth dorsal plates to each other as 36: 31: 34: 42: 43: 41.

Head conspicuously wider than long (9: 8), widely rounded in front of eyes; sides well rounded caudad of eyes. Hairs of moderate length and sparse, as usual.

Antennae short and moderately attenuated. Articles beyond the second short and very short, decreasing distad. Clothed as usual.

Eyes composed of about 6 ocelli arranged in two series:— thus, 1+3, 2. Ocelli distinct, regular in form and arrangement.

Prosternum 1.6 times wider than long. Distance between chitinous spots 2.1 times width at median incision; 3.5 times the dental line. Median incision wide and shallow, strictly semicircular in form. Teeth small, the outer of each pair rather larger than the inner, the interval between the two well rounded; line of apices strongly re-curved. Spine contiguous with base of outer tooth, fine. Lateral

margin of anterior portion beginning at spine and sloping ectocaudad; a little convex, bent ectad near caudal end for a short distance.

First dorsal plate 1.72 times wider than long; sides convex, widest a little in front of middle of length. Posterior angles of thirteenth plate a little produced; those of the eleventh slightly more so. Caudal margin of other minor plates more or less bent forwards at ends; or corners of the ninth obliquely excised.

Coxal pores small and circular; 2, 2, 2, 2.

Spines of first to third legs inclusive, $\frac{0, 0, 0, 0, 1}{0, 0, 0, 0, 0}$; of fourth and fifth, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 0, 1}$; of sixth, $\frac{0, 0, 0, 1, 2}{0, 0, 0, 0, 1}$; of seventh and eighth, $\frac{0, 0, 0, 1, 2}{0, 0, 0, 1, 1}$; of tenth and eleventh, $\frac{0, 0, 0, 2, 2}{0, 0, 0, 1, 1}$; of twelfth, $\frac{0, 0, 1, 1, 2}{0, 0, 0, 1, 1}$; of thirteenth, $\frac{0, 0, 2, 1, 0}{0, 0, 2, 1, 1}$; of penult, $\frac{0, 0, 2, 0, 0}{0, 1, 2, 0, 1}$, claws; of the anal, $\frac{0, 0, 2, 0, 0}{0, 1, 1, 1, 0}$, armed with two claws.

In the male the anal legs are short and moderately, uniformly inflated. The penult legs are similar excepting for the tibial lobe; lobe a relatively slender and moderately long cylindric process which widens at base but distally is rounded and not at all expanded; distal hairs straight and divergent.

Length of type 6.5 mm.

LOCALITY.—South Carolina (Landrum! August, 1910).

NAMPABIUS TENNESSEENSIS, sp. nov.

DIAGNOSIS.—Dorsal spines of anal and penult legs 0, 0, 2, 1, 0; ventral, 0, 1, 1, 1, 0. Dorsal spines of twelfth legs, 0, 0, 2, 1, 0. Dorsal spines of tenth and eleventh legs (as also of the first to ninth), 0, 0, 0, 1, 1. Ventral spines of ninth to twelfth legs (as also of the fifth or sixth to eighth), 0, 0, 0, 1, 1.

Ocelli 8 or 9 in two or three series.

Prosternum 1.54 times wider than long. Distance between chitinous spots $2\frac{1}{2}$ times width at median incision; 3.5–3.8 times the dental line.

Body eight times longer than width of tenth plate.

Head wider than long.

Process of tibia of penult legs of male flattened in a distoectal proximomesal direction; considerably longer along base than the height above same; expanded at distal end. (Plate 3, fig. 3–4).

Dorsum pale chestnut. Head darker chestnut, shining.

DESCRIPTION.—Dorsum pale chestnut. Head a deeper, shining

chestnut. Antennae chestnut, excepting the tips which are lighter. Prosternum rather more brownish than the head, with the prehenso-rial feet lighter and at tips somewhat light ferruginous. Venter pale; the anterior plates suffused with purplish; the caudal plates burnt brown. Legs pale, excepting the posterior pairs which are brown or brownish orange.

Body moderate, in the male type being near eight times longer than wide. Widths of head and of first, third, eighth, tenth, and twelfth plates to each other as 36:30:32:41:42:40.

Head wider than long in ratio 12:11; not varying much in width between eyes and level of marginal breaks; broadly rounded cephalad. Hairs short and sparse.

Antennae short as in related species. Moderately attenuated. Articles short, decreasing distad. Hairs on proximal articles rather sparse, becoming denser distad.

Eyes composed of eight or nine ocelli arranged in two or three series:—thus, 1+4, 3; 1+3, 3, 2; 1+4, 3, 1.

Prosternum 1.54 times wider than long. Distance between chitinous spots $2\frac{1}{2}$ times the width at level of bottom of median incision; 3.8–3.5 times the dental line. Median incision wide and shallow, sides strongly converging, rounded at bottom. Teeth moderately small, acute; line of bases forming a strongly reentrant angle and the line of apices accordingly recurved. Sides of anterior portion sloping immediately from the outer teeth, straight, slanting more strongly ectad than usual.

First dorsal plate widest near the middle, from where the sides are subparallel cephalad and rather strongly converging caudad. Caudal angles of eleventh and thirteenth dorsal plates a little produced in the typical manner; those of the ninth straight or a little oblique.

Coxal pores small and circular, the proximal ones in each series somewhat smaller than the distal; 2, 2, 2, 2 or 2, 3, 3, 2.

Spines of first legs, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 0, 0}$; of the second, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 0, 0}$; of the third and fourth, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 0, 1}$; of the fifth, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 0, 1}$ or $\frac{0, 0, 0, 1, 1}{0, 0, 0, 1, 1}$; of the sixth to eleventh, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 1, 1}$; of the twelfth, $\frac{0, 0, 2, 1, 0}{0, 0, 0, 1, 1}$; of the thirteenth, $\frac{0, 0, 2, 1, 0}{0, 0, 1, 1, 1}$; of the penult, $\frac{0, 0, 2, 0, 0}{0, 1, 1, 1, 0}$, claws 2; of the anal, $\frac{0, 0, 2, 0, 0}{0, 1, 1, 1, 0}$, claws 2.

Anal legs of the male short; uniformly and but little thickened. Penult legs as usual; lobe or process extending dorsocaudo-distad, flattened in a distoectal and proximo-mesal direction, considerably

longer along line of attachment than its height above surface of joint, at distal end expanded over proximal portion, hairs at distal end of process as usual. (Plate 3, fig. 3-4).

Gonopods of male broad but very short, rounded; bearing two bristles.

Length of male type, 6.25 mm.; width of tenth plate, .78 mm.; length of antennae 1.75 mm.; of anal legs, 2 mm.

LOCALITY.—Tennessee (Russellville!).

NAMPABIUS LONGICEPS, sp. nov.

DIAGNOSIS.—Dorsal spines of anal and penult legs 0, 0, 2, 0, 0; ventral, 0, 1, 1, 1, 0. Dorsal spines of thirteenth legs, 0, 0, 2, 1, 0; ventral 0, 0, 1, 1, 1. Dorsal spines of twelfth legs 0, 2, 1, 1—0, 0, 1, 1, 1. Dorsal spines of eleventh legs, 0, 0, 0, 0, 1; of tenth (as also of the second to ninth), 0, 0, 0, 1, 1. Ventral spines of ninth to twelfth pairs of legs (as also of the fourth or fifth to eighth), 0, 0, 0, 1, 1.

Ocelli 7 or 8 in two series.

Prosternum about 1.48 times wider than long. Distance between chitinous spots 2.15 times the width at median incision; 3.5 times the dental line.

Median lobe of claw of female gonopods but little longer than the lateral. Spines wide with the sides parallel from base to the very short, acuminate distal portion.

Body slender, being 8.5 times longer than the width of the tenth plate.

Head only slightly wider than long, less rotund than in *N. lulae*.

First dorsal plate 1.8+ times wider than long.

Dorsum testaceous with anterior and posterior plates darker. Head reddish or chestnut.

DESCRIPTION.—Dorsum testaceous, with the anterior and the posterior plates darker. Head reddish or chestnut. Antennae brown, pale at tips. Prosternum brown. Venter very pale, with the caudal plates darker and the anterior ones strongly suffused with violaceous. Legs pale like the venter, the posterior pairs darker brown.

Body comparatively slender being about 8.5 times longer than width of tenth plate. Widths of head and of first, third, eighth, tenth, and twelfth plates to each other as 36: 30: 33: 41: 41: 39.

Head only slightly wider than long (36: 35); less rotund than in *lulae*. Hairs moderate in length, few.

Antennae very short, reaching only the fourth or fifth segment. Articles short and very short, decreasing distad as usual. Hairs of moderate length, rather sparse proximally, becoming more dense distad.

Ocelli 7 or 8; arranged in two series:—thus, 1+3, 3; 1+4, 3.

Prosternum 1.48— times wider than long. Distance between chitinous spots 2.15 times the width at level of median incision; 3.5 times as great as the dental line. Teeth subequal, acute, with sides nearly straight but with interval on each side narrowly rounded at the bottom; line of apices recurved. Median incision well rounded at bottom as usual; but the sides more nearly straight and less converging. Sides of anterior portion but slightly convex, sloping ecto-caudad immediately from base of spine.

First dorsal plate widest near anterior end; the sides moderately converging caudad; 1.8 times wider than long. Thirteenth plate with caudal angles a little produced; but the eleventh and ninth with posterior margin straight all the way across or nearly so, or the eleventh showing a tendency for the angles to be weakly produced.

Coxal pores very small; 2, 3, 3, 3.

Spines of the first legs, $\frac{0, 0, 0, 0, 1}{0, 0, 0, 0, 0}$; of the second, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 0, 0}$; of the third, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 0, 1}$; of the fourth, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 0, 1}$ or $\frac{0, 0, 0, 1, 1}{0, 0, 0, 1, 1}$; of the fifth to tenth, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 0, 1}$; of the eleventh, $\frac{0, 0, 0, 0, 1}{0, 0, 0, 0, 1}$; of the twelfth, $\frac{0, 0, 2, 1, 1}{0, 0, 0, 1, 1}$ or $\frac{0, 0, 1, 1, 1}{0, 0, 0, 1, 1}$; of the thirteenth, $\frac{0, 0, 2, 1, 0}{0, 0, 1, 1, 1}$; of the penult and anal, $\frac{0, 0, 2, 0, 0}{0, 1, 1, 1, 0}$, with two claws.

Claw of the gonopods of female short, moderately curved, tripartite; lobes short and acute, the median being but little longer than the lateral. Basal spines wide with sides parallel from base to the acuminate distal portion which is relatively very short with its straight sides converging to an almost obtuse angle, its outer side being longer than its inner. Inner side of first article of gonopods a little convex distally and slightly concave proximally, subparallel with its fellow.

Length of type 6.5 mm.; of antenna, 1.9 mm.; of anal leg, 1.8 mm.; width of tenth dorsal plate, .76 mm.

LOCALITY.—North Carolina (Asheville!).

NAMPABIUS LULAE, sp. nov.

DIAGNOSIS.—Dorsal spines of anal and penult legs, 0, 0, 2, 0, 0; ventral, 0, 1, 1, 1, 0. Dorsal spines of thirteenth legs, 0, 0, 2, 1, 1; ventral, 0, 0, 0, 1, 1—0, 0, 1, 1, 1. Dorsal spines of twelfth legs,

0, 0, 2, 1, 1; of the eleventh, 0, 0, 0, 0, 1; of the tenth (as also of second to ninth), 0, 0, 0, 1, 1. Ventral spines of ninth to twelfth pairs of legs (as also of the sixth to eighth), 0, 0, 0, 1, 1.

Ocelli 7 to 11 in 2 or 3 series.

Prosternum 1.55+ times wider than long. Distance between chitinous spots 2.1 times the width at median incision; 3.5 times the dental line.

Body about seven times longer than the width of tenth plate.

Head subrotund; wider than long.

First dorsal plate 1.75 times wider than long.

Dorsum rather dark brown, in some of reddish caste. Head deep reddish brown or almost black.

DESCRIPTION.—Dorsum rather dark brown, which may be somewhat reddish in caste. Head deep reddish brown or almost black. Antennae dark brown proximally, pale at tips. Prosternum brown, the prehensorial feet considerably lighter in shade. Venter dilute greyish brown to light brown, the caudal plates darker brown as usual and the anterior segments commonly distinctly suffused with violaceous. Legs like corresponding portion of venter, the posterior pairs being thus darker, brown, paler distally.

Body seven times, a little more or less, longer than the width of the tenth plate. Widths of head and of first, third, eighth, tenth, and twelfth plates to each other as 37:31:34:42:44:42.

Head subrotund, wider than long in ratio 35.5:33.5. Hairs moderately short, sparse.

Antennae short, only moderately attenuated. Articles short, decreasing distad as usual. Hairs of moderate length, not especially dense.

Eyes each composed of from 7 to 12 ocelli in two or three series:—thus, 1+4, 4, 2; 1+4, 4, 1; 1+3, 3; 1+4, 3. Single ocellus relatively large.

Prosternum 1.55+ times wider than long. Distance between chitinous spots 2.1 times width at level of bottom of median incision; 3.5 times the dental line. Teeth small and acute; line of apices clearly recurved; interval between teeth of each pair angular. Median incision relatively wide, semicircular, being widely curved at bottom and with sides considerably converging. Spine moderately stout at base, long and acutely, finely acuminate as usual; parallel with ectal edge of outer tooth. Lateral slopes beginning at spine and extending directly ectocaudad.

First dorsal plate 1.75 times wider than long; sides from middle to anterior end nearly straight and parallel; caudad of middle strongly

convexly rounding in mesad about the caudal corners. Ninth plate with its caudal margin straight or a little bent forwards at ends. The eleventh and thirteenth plates with posterior corners only slightly produced caudad.

Coxal pores small; on distal portion of coxa; in number 2, 2, 2, 2, mostly.

Spines of first legs, $\frac{0}{0}, \frac{0}{0}, \frac{0}{0}, \frac{0}{0}, \frac{1}{0}$; of second, $\frac{0}{0}, \frac{0}{0}, \frac{0}{0}, \frac{1}{0}, \frac{1}{0}$; of third to tenth, $\frac{0}{0}, \frac{0}{0}, \frac{0}{0}, \frac{1}{1}, \frac{1}{1}$, the dorsal spines on tenth legs being on anterior side of joint; of twelfth, $\frac{0}{0}, \frac{0}{0}, \frac{2}{0}, \frac{1}{1}, \frac{1}{1}$, the dorsal spine of fourth article being posterior and that of fifth joint anterior in position; of thirteenth, $\frac{0}{0}, \frac{0}{0}, \frac{2}{0}, \frac{1}{1}, \frac{1}{1} - \frac{0}{0}, \frac{0}{0}, \frac{2}{0}, \frac{1}{1}, \frac{1}{1}$, the two single dorsal spines situated on posterior side; of penult and anal legs, $\frac{0}{0}, \frac{0}{0}, \frac{2}{0}, \frac{0}{0}, \frac{0}{0}, \frac{0}{0}, \frac{1}{1}, \frac{1}{1}, \frac{0}{0}$ each with two claws.

Anal and penult legs of male short and moderately, uniformly inflated; fourth joint longitudinally sulcate above, the third and fifth less distinctly so. Process of penult legs present in usual position.

Gonopods of male each extending somewhat ectocaudad, the mesal side being very long and convex and the ectal relatively very short, bearing two (or one) bristles.

Length from 5 to 6.5 mm. A male 6 mm. long has antenna 2 mm. long; anal leg 1.8 or 1.9 mm. long; and the tenth plate .87 mm. wide.

TYPE LOCALITY.— Georgia (Lula).

KNOWN LOCALITIES.— Georgia (Lula!); Tennessee (Johnson City! Russellville!).

NAMPABIUS LUNDII (MEINERT).

Lithobius lundii Meinert, Myriap. Mus. Haumensis, 1886, 3, p. 12.

DESCRIPTION.— The original description, somewhat rearranged, is as follows:

Brunneus, capite cum antennis obscuriore, subtus pallidiore, pedibus flavis.

Sat gracilis, sublaevis.

Capite suborbiculare, fere aequae longo ac lato.

Antennae breviusculae, 19-articulatae, articulis longiusculis.

Oculi ocellis 6-7, in series 3 digestis.

Dentes prosternales bini.

Laminae dorsales omnes angulis rectis.

Pori coxales 2, 3, 3, 2, rotundi, parvi vel perparvi.

Pedes corporis primi paris calcaribus 0, 0, 1; pedes anales calcari-
bus 1, 1, 1, 0 armati, unguibus binis; pedes penultimi paris unguibus
binis armati. Pedum analium coxae inermes.

Pedum penultimorum tibiae in angulo interno appendice parvo
nodiformi instructae.

Pedes postici breviusculi, crassiusculi.

Long. (maris) 6.5 mm.

LOCALITY.—Near New York City (L. Lund): the type is in the
Museum at Copenhagen.

GARIBIUS, gen. nov.

Body considerably narrowed from the tenth segment cephalad to the
first, the dorsal plate of which is much narrower than the head a
little narrower than that of the third segment. Moderate to slender,
the length varying from 7.5 to 9.5 times the width of the tenth plate.
Varying from 5 to 9.5 mm. in length.

Antennae short; uniformly composed of twenty articles.

Ocelli in three or, less commonly, in two series; 6 to 14 in number.
Single ocellus typically not larger than the others.

Prosternal teeth 2+2; line of apices straight or but little recurved.
Median incision v-shaped, strictly angular at bottom.

Posterior angles of none of the dorsal plates produced.

Claw of the female gonopods in all known species tripartite. Basal
spines 2+2, slender. Mesal side of first article nearly straight.

Coxal pores small and circular; uniseriate; from 2, 3, 3, 2 to 3, 4, 4, 3.

Dorsal spines of the anal legs 1, 0, 2, 0, 0 or rarely 1, 0, 2, 1, 0; ven-
tral, 0, 1, 3, 1, 0; claw 1. Dorsal spines of penult legs 1, 0, 2, 1, 0 or
1, 0, 2, 1, 1; ventral, 0, 1, 3, 2, 1; claws 3. Dorsal spines of thirteenth
legs, 1, 0, 2, 1, 1; ventral, 0, 1, 3, 3, 2 to 0, 0, 2, 3, 2. Dorsal spines of
twelfth legs, 1, 0, 2, 1, 1 or 0, 0, 0, 2, 2 to 1, 0, 2, 1, 2. Dorsal spines of
eleventh legs 0, 0, 2, 1, 1 or 0, 0, 0, 2, 2 to 1, 0, 2, 1, 2; ventral, 0, 0, 0,
2, 1 to 0, 0, 2, 3, 2. Dorsal spines of first legs 0, 0, 1, 1, 1 to 0, 0, 2, 2, 1;
ventral, 0, 0, 0, 2, 1 to 0, 0, 1, 2, 1. Last three or four pairs of coxae
thus dorsally armed; while rarely none of the coxae are laterally
armed, in most those of the last pair are, and those of the last two
pair may be.

Anal legs of male moderately thickened; tibia always bearing at
distal end on dorsomesal side a low crest bearing sensory setae

(Plate 4, fig. 6, 7, 9) much as in the genus *Nadabius* (*collium* Chamb., *iowensis* Mein., and allies). The tibia of the penult legs may or may not show a more weakly developed similar crest.

TYPE.—*G. monticolens*, sp. nov.

DISTRIBUTION.—Southeastern States and northward to Pennsylvania and New Jersey.

These forms seem particularly to prefer to live under the bark of fallen trees.

NOTE.—In my paper on the Lithobiomorpha of the Southeastern States (Ann. Ent. soc. America, 1911, 4, p. 32–50) several of the species now described were placed together under the name *Lithobius branneri* Bollman, the several forms being considered tentatively as varieties.

The following key is given as an aid in the identification of the species.

Key to species of Garibius.

- a. Dorsal spines of anal legs 1, 0, 2, 1, 0. *G. catawbae*, sp. nov.
- aa. Dorsal spines of anal legs 1, 0, 2, 0, 0.
 - b. First seven or more pairs of legs with the third joint ventrally unarmed.
 - c. Third joint of all legs dorsally armed; none of the coxae laterally armed; dorsal spines of penult legs 1, 0, 2, 1, 0; of the twelfth, 0, 0, 2, 1, 1 or 1, 0, 2, 1, 1. *G. georgiae*, sp. nov.
 - cc. Third joint of first ten or more pairs of legs dorsally unarmed; last pair of coxae laterally armed; dorsal spines of penult legs 1, 0, 2, 1, 1; of the twelfth 0, 0, 2, 2, 2.
 - G. alabamiae*, sp. nov.
 - bb. All legs, or all excepting the first pair, with the third joint armed both dorsally and ventrally.
 - c. Last two pairs of coxae laterally armed.
 - d. Ventral spines of second to sixth legs 1, 3, 1; of 1st, 1, 2, 1, with the dorsal 2, 1, 1; head equal in length and breadth. *G. pagoketes*, sp. nov.
 - dd. Ventral spines of second to seventh legs 1, 2, 1; of first 0, 2, 1, with the dorsal 1, 1, 1; head distinctly wider than long. *G. mississippiensis*, sp. nov.
 - cc. Either none, or only the last pair, of coxae laterally armed.
 - d. None of the coxae laterally armed; dorsal spines of the eleventh legs 2, 1, 2, the ventral 1, 3, 2; ventral spines of the first legs 1, 3, 2. *G. dendrophilus*, sp. nov.

- dd.* Last pair of coxae laterally armed; dorsal spines of eleventh legs 2, 1, 1, ventral 2, 3, 2 or 3, 3, 2; ventral spines of first legs, 1, 2, 1 or 1, 3, 1.
- e.* Ventral spines of eleventh and twelfth legs 2, 3, 2; of second to sixth, 1, 3, 2. *G. opicolens*, sp. nov.
- ee.* Ventral spines of eleventh and twelfth legs 3, 3, 2; of second to sixth 1, 3, 1. *G. monticolens*, sp. nov.

GARIBIUS CATAWBAE, sp. nov.

DIAGNOSIS.—Dorsal spines of anal and of penult legs, 1, 0, 2, 1, 0. Ventral spines of thirteenth legs, 1, 3, 3, 2 or 1, 2, 3, 2. Dorsal spines of twelfth legs, 0, 0, 2, 1, 1; ventral, 0, 0, 2, 3, 2. Dorsal spines of eleventh legs, 0, 0, 2, 2, 2; ventral, 0, 0, 2, 3, 2. Dorsal spines of first legs, 0, 0, 2, 1, 1; ventral, 0, 0, 1, 2, 1. Third joint of all legs dorsally and also ventrally armed. Last pair of coxae laterally armed.

Antennae as usual.

Ocelli about 14 in three series.

Prosternum 1.65 times wider than long. Distance between chitinous spots 2.7 times the width at median incision; 3.78 times the dental line. Median incision strictly V-shaped.

Coxal pores 3, 4, 4, 3.

Body about 8.4 times longer than width of tenth plate.

Length (of type) 8 mm.

First dorsal plate 1.73 times wider than long.

Anal legs in the male with the usual crest. (Plate 4, fig. 8-9). Penult legs with the tibia also bearing a sharp longitudinal ridge along dorsocaudal side the form of which at its distal end is somewhat suggestive of the crest of the anal leg.

Dorsum light chestnut; head scarcely darker.

DESCRIPTION.—Light chestnut above. Head scarcely darker. Antennae in color like the head, pale at tips.

Prosternum light brown, with the prehensorial feet paler. Venter pale, the caudal plates darker, burnt brown. Legs pale, with tarsi most pigmented; posterior pairs darker, brown or pale chestnut, lighter distally.

Body of usual form. Length about 8.4 times width of the tenth dorsal plate. Widths of head and of first, third, eighth, tenth, and twelfth plates to each other as 45: 40: 42: 49: 50: 44.

Head of equal width and length or very nearly so. Nearly of uni-

form width between eyes and marginal breaks; narrowed rather more than usual cephalad.

Antennae short. Composed of the usual twenty articles which are short, articles more loosely united distally.

Eyes composed of about 14 ocelli arranged in three series:— thus, 1+5, 4, 4.

Prosternum 1.65 times wider than long. Distance between chitinous spots 2.7 times width at level of bottom of median incision; 3.78 times the length of dental line. Mesal incision strictly V-shaped, the sides being straight and meeting in an acute angle at bottom. Teeth acute, with their sides straight; the interval between each pair subacute at bottom or but narrowly rounded; inner tooth distinctly larger than the outer; line of apices straight or very nearly so. Lateral slope beginning at outer tooth; convex for entire length, most strongly so near its middle.

First dorsal plate with sides convex anteriorly; widest a little back of anterior end from where to the rounded caudal corners the sides are nearly straight and converge conspicuously; about 1.73 times wider than long. Posterior corners of ninth, eleventh, and thirteenth dorsal plates simply rounded.

Coxal pores small, regularly decreasing proximad on each coxa; in number, 3, 4, 4, 3.

Spines of first legs, $\frac{0, 0, 2, 1, 1}{0, 0, 1, 2, 1}$; of the second to sixth, $\frac{0, 0, 2, 2, 1}{0, 0, 1, 3, 1}$; of seventh, $\frac{0, 0, 2, 2, 2}{0, 0, 1, 3, 1}$; of eighth, $\frac{0, 0, 2, 2, 2}{0, 0, 1, 3, 1}$ or $\frac{0, 0, 2, 2, 2}{0, 0, 1, 3, 2}$; of ninth, $\frac{0, 0, 2, 2, 2}{0, 0, 1, 3, 2}$; of tenth and eleventh, $\frac{0, 0, 2, 2, 2}{0, 0, 2, 3, 2}$; of twelfth, $\frac{0, 0, 2, 1, 1}{0, 0, 2, 3, 2}$; of thirteenth, $\frac{1, 0, 2, 1, 1}{0, 1, 3, 3, 2}$ or $\frac{1, 0, 2, 1, 1}{0, 1, 2, 3, 2}$; of penult, $\frac{1, 0, 2, 1, 0}{0, 1, 3, 2, 1}$, claws 3; of the anal, $\frac{1, 0, 2, 1, 0}{0, 1, 3, 1, 0}$; claw single. Coxae of anal legs laterally armed.

Anal legs of male moderately crassate, with the tarsi more or less abruptly more slender than the tibia, tibia at distal end with dorsomesal corner flattened and there bearing the typical low ridge or crest. Dorsomesal edge of crest forming almost a straight line, at distal end convex; bearing a number of moderately long hairs. (Plate 4, fig. 8-9). Joints longitudinally sulcate above and laterally. Penult legs with tibia bearing a sharp longitudinal ridge along dorsocaudal side, this at distal end in appearance suggestive of the crest of anal legs.

Length of type (♂) 8 mm.; length of antennae, 2.5 mm.; of anal legs also near 2.5 mm.; width of tenth plate, .95 mm.

LOCALITY.— North Carolina (Catawba!).

GARIBIUS MONTICOLENS, sp. nov.

DIAGNOSIS.—Dorsal spines of anal legs 1, 0, 2, 0, 0. Dorsal spines of penult legs 1, 0, 2, 1, 0. Ventral spines of thirteenth legs, 0, 1, 3, 3, 2. Dorsal spines of twelfth legs, 1, 0, 2, 1, 1; ventral, 0, 0, 3, 3, 2. Dorsal spines of eleventh legs, 0, 0, 2, 1, 1; ventral, 0, 0, 3, 3, 2. Dorsal spines of first legs, 0, 0, 2, 2, 1; ventral, 0, 0, 1, 2, 1. All legs with third joint armed both dorsally and ventrally. Last pair of coxae laterally armed.

Antennae of usual form. (Plate 4, fig. 3).

Ocelli about 14 in three series. (Plate 4, fig. 4).

Prosternum 1.53 times wider than long. Distance between chitinous spots 2.38 or 2.4 times width at median incision; 3.58 times length of dental line. (Plate 4, fig. 1-2).

Anal leg of male moderately inflated; tarsi abruptly more slender; tibia bearing a crest in the usual position, this clothed with a subdense series of long hairs. (Plate 4, fig. 6-7). Penult legs without lobes.

Body slender; 9.5 times as long as width of tenth plate.

Head cordate; equal in length and width or nearly so.

First dorsal plate 2.5 to 2.6 times wider than long.

Dorsum light brown. Head dilute ferruginous brown or somewhat orange with frontal region paler.

DESCRIPTION.—Above, light brown. Head dilute ferruginous brown or somewhat orange, with the frontal region paler. Antennae light brown, paler distad as usual. Prosternum lighter than head. Venter pale testaceous; the caudal plates more deeply pigmented, more or less orange. Legs pale testaceous with the tarsi more pigmented, the posterior pairs darker, brownish.

Body slender, being nearly 9.5 times longer than the width of the tenth plate. Widths of head and of first, third, eighth, tenth, and twelfth plates to each other as 54:46:47:54:56:55.

Head cordate. Equal in length and width or very nearly so. Nearly constant in width between level of eyes and that of lateral breaks or a little caudad of then.

Antennae short. Composed of the usual twenty articles, these being loosely joined. (Plate 4, fig. 3).

Eyes consisting of about 14 ocelli arranged in three series:—thus, 1+5, 5, 3. Single ocellus oval. Ocelli of upper series, especially the more caudal ones, conspicuously larger than the other ones, the ocelli decreasing in size cephalad and ventrad. (Plate 4, fig. 4).

Prosternum 1.53 times wider than long. Distance between chitinous spots 2.38–2.4 times width at level of bottom of median incision; 3.58 times length of dental line. Mesal incision v-shaped, with sides nearly straight. Teeth acute, the mesal one larger than the ectal; line of apices straight or slightly recurved. Spine considerably stouter at base than the hairs, slender distally, of moderate length. Lateral slopes convex cephalad, concave and bending ectad caudally. (Plate 4, fig. 1–2).

First dorsal plate widest a little caudad of the anterior end from where the sides are nearly straight and moderately converging to the rounded posterior corners; 2.5 to 2.6 times wider than long. Posterior angles of ninth, eleventh, and thirteenth dorsal plates simply rounded, with no tendency toward production.

Coxal pores small and circular; on each coxa decreasing regularly proximad.

Spines of first legs, $\frac{0, 0, 2, 2, 1}{0, 0, 1, 2, 1}$; of second to fifth, $\frac{0, 0, 2, 2, 1}{0, 0, 1, 3, 1}$; of sixth, $\frac{0, 0, 2, 2, 1}{0, 0, 1, 3, 1}$ or $\frac{0, 0, 2, 2, 2}{0, 0, 1, 3, 1}$; of seventh, $\frac{0, 0, 2, 2, 2}{0, 0, 1, 3, 2}$; of eighth, $\frac{0, 0, 2, 2, 2}{0, 0, 1, 3, 2}$ or $\frac{0, 0, 2, 2, 2}{0, 0, 2, 3, 2}$; of ninth and tenth, $\frac{0, 0, 2, 2, 2}{0, 0, 2, 3, 2}$; of the eleventh, $\frac{0, 0, 2, 1, 1}{0, 0, 3, 3, 2}$; of the twelfth, $\frac{1, 0, 2, 1, 1}{0, 0, 3, 3, 2}$; of the thirteenth, $\frac{1, 0, 2, 1, 1}{0, 1, 3, 3, 2}$; of the penult, $\frac{1, 0, 2, 1, 0}{0, 1, 3, 2, 1}$, with three claws, of which the anterior is often very small, sometimes spine of coxa is doubled, making the dorsal formula thus 2, 0, 2, 1, 0; of the anal, $\frac{1, 0, 2, 0, 0}{0, 1, 3, 1, 0}$, the claw single, long.

Anal legs of male moderate in length, moderately inflated distad to the tarsus which is abruptly more slender; tibia obliquely complanate at distal end on mesal side toward dorsal surface and there bearing a low subtriangular crest, the elevated end of which is caudad, its mesal, gently convex edge gradually resuming the level of the joint proximad, the crest bearing a subdense series of moderately long hairs which tend to be somewhat curved caudad at their distal ends (Plate 4, fig. 6–7). Penult legs more slightly thickened, without special lobes. Third, fourth, and fifth joints of both anal and penult legs longitudinally sulcate dorsally.

Length of male type 9.5 mm.; width of tenth plate 1.07 mm.; length of antenna, 3.5 mm.; of anal leg also 3.5 mm.

TYPE LOCALITY.—Tennessee (Unaka Springs!).

LOCALITIES.—Tennessee (Unaka Springs!); North Carolina (Ashville! Brown's Summit!).

GARIBIUS OPICOLENS, sp. nov.

DIAGNOSIS.—Dorsal spines of anal legs 1, 0, 2, 0, 0; of penult 1, 0, 2, 1, 0. Ventral spines of thirteenth legs, 0, 1, 3, 3, 2. Dorsal spines of twelfth legs, 1, 0, 2, 1, 1, ventral, 0, 0, 2, 3, 2. Dorsal spines of eleventh legs, 1, 0, 2, 1, 1; ventral, 0, 0, 2, 3, 2. Dorsal spines of first legs, 0, 0, 2, 2, 1; ventral, 0, 0, 1, 2, 1, or 0, 0, 1, 3, 1. Third joint of all legs armed both dorsally and ventrally. Last pair of coxae laterally armed.

Brown to light chestnut; head subferruginous, dusky caudad of suture.

Body moderate, being about 8.25 times longer than width of tenth plate.

Head broadly subcordate, nearly equal in length and breadth.

Eyes composed of from thirteen to fifteen ocelli in three series.

Prosternum 1.54 times wider than long, as in *G. monticolens*.

Penult legs without lobes; anal legs moderately inflated with the tarsi abruptly more slender, and the tibia bearing the usual crest which is nearly as in *monticolens*.

Gonopods of female with claw short and wide, tripartite, outermost lobe smallest; outer spine considerably longer than the inner on each side, sides of spines subparallel to the short and abruptly acuminate distal portion.

DESCRIPTION.—Dorsum brown to light chestnut, with nearly always a blackish median longitudinal stripe for all or most of length while two or more of the most anterior plates may be margined, caudally with blackish; fifteenth plate abruptly lighter, yellowish or light orange. Head subferruginous, dusky caudad of suture with a blackish median spot or stripe on caudal portion. Antennae dark proximally, orange or rufous distad. Prosternum orange or light ferruginous. Venter brown, the last plate orange like the corresponding dorsal one, with the immediately preceding one or two also often lighter. The posterior pairs of legs more densely pigmented as usual, the distal joints being light orange.

Body of moderate build, being about 8.23 to 8.26 times longer than width of tenth dorsal plate.

Head broadly subcordate. Equal in length and breadth or very slightly longer (49:48). Widest just caudad of the marginal breaks from where the sides converge slightly cephalad to the eyes.

Antennae short. The twenty articles short, of uniform proportions,

moderately compactly joined, being less loose than in types of *monticolens*; ultimate article long and pointed. Hairs rather long, straight.

Eyes composed mostly of from thirteen to fifteen ocelli arranged in three series:—thus, 1+5, 4, 3; 1+5, 4, 4; 1+5, 5, 4. The single ocellus is typically broad and may be materially the largest; of the others those of the dorsal series are materially the largest, these decreasing in size cephalad.

Prosternum cir. 1.54 times wider than long. Distance between chitinous spots 2.4 times width at level of median incision; 3.78+ times the dental line. Outer tooth of each pair smaller than the inner one, both subacute; line of apices very slightly recurved. Interval between teeth of each pair semicircular. Sides of median incision straight, the bottom well rounded. Ectal spine materially stouter at base than neighboring bristles, rapidly acuminate to about middle of length, distad of where it is bristle-like; straight and moderately long.

Coxal pores circular and small; nearly uniform in size or the most proximal on coxa reduced. Common formulae are these: 3, 4, 4, 3; 4, 4, 4, 3; 4, 5, 4, 3; 4, 5, 4, 4; 4, 5, 5, 4.

Spines of first legs, $\frac{0, 0, 2, 2, 1}{0, 0, 1, 2, 1}$ or $\frac{0, 0, 2, 2, 1}{0, 0, 1, 3, 1}$; of the second to sixth pairs, $\frac{0, 0, 2, 2, 1}{0, 0, 1, 3, 2}$; of the seventh, $\frac{0, 0, 2, 2, 2}{0, 0, 1, 3, 2}$; of the eighth to tenth, $\frac{0, 0, 2, 2, 2}{0, 0, 2, 3, 2}$; of the eleventh and twelfth, $\frac{1, 0, 2, 1, 1}{0, 0, 2, 3, 2}$; of the thirteenth, $\frac{1, 0, 2, 1, 1}{0, 1, 3, 2, 1}$; of the penult, $\frac{1, 0, 2, 1, 0}{0, 1, 3, 2, 1}$, with three claws, of which the accessory ones are small but distinct; of the anal, $\frac{1, 0, 2, 0, 0}{0, 1, 3, 1, 0}$, claw single, long. The median ventral spines of legs especially long. In one specimen the inner ventral spine of prefemur of both penult legs is bifid for about half of length. Coxae of anal legs laterally armed.

Anal legs of male of moderate length, considerably longer than the penult; moderately inflated, with the tibia a little clavately widening distad and the tarsal joints abruptly more slender; tibia at distal end with dorsomesal surface obliquely complanate and there bearing the usual crest, the free edge of which toward caudal end is convex and somewhat lowering distad but materially less so than the general surface of joint and sides of crest; crest bearing a series of hairs which are straight or with some of them a little bent caudad at ends, these hairs more slender than bristles of other parts of joint; the crest very similar to that of *monticolens*, see Plate 4, fig. 6-7. Penult leg of male also moderately inflated, without any crest or special lobe. Prefemur, femur and, less distinctly, the tibia of anal and penult legs longitudinally dorsally sulcate.

The single article of the gonopods of male small but well exposed. Distally obliquely subtruncate. Bearing on distal surface mostly two long bristles.

Claw of female gonopods short and relatively wide; tripartite, the divisions acute and rather longer than usual, the middle lobe but slightly longer than the inner one, the outer one considerably shorter and more slender. Basal spines with outer one of each pair conspicuously longer than the inner; sides of spines subparallel or slightly diverging from base to the acuminate distal portion which is very short, occupying about one fourth of the total length, its sides strongly converging, its apex narrowly rounded; a denticle usually manifest on margin at beginning of acuminate portion on each side.

Length 8 to 11 mm. A specimen 10.5 mm. in length has antennae 2.6 mm. long and anal legs 3.5+ mm. long.

Immaturus.— Body pale yellow or dilute brown tinged with violaceous. Head yellow or pale orange. Anal legs whitish to yellow. Antennae pale yellow.

Antennae very short. Articles twenty, these distad of the fourth or fifth being very short, proportionately more so than in the adult, closely compacted. Hairs long, not dense.

Eyes composed of six to eight ocelli arranged in two series:— thus, 1+3, 2; 1+3, 3; 1+4, 3. Ocelli pale. Single ocellus somewhat largest; caudal two of dorsal row large, subequal the most anterior and those of the lower row reduced.

Prosternum practically as in the adult as regards the form and relations of teeth and ectal spines. Teeth pale.

Coxal pores small, with the most proximal one on each coxa only very slightly smaller than the distal one; 2, 2, 2, 2.

Spines of first legs, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 0, 1}$, $\frac{0, 0, 0, 0, 1}{0, 0, 0, 0, 1}$ or $\frac{0, 0, 0, 1, 1}{0, 0, 0, 1, 1}$; of the second to fifth pair, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 1, 1}$; or $\frac{0, 0, 1, 2, 1}{0, 0, 0, 1, 1}$; of the sixth, $\frac{0, 0, 1, 2, 1}{0, 0, 0, 2, 1}$ to $\frac{0, 0, 1, 1, 1}{0, 0, 0, 1, 1}$; of the seventh, $\frac{0, 0, 1, 1, 1}{0, 0, 0, 1, 1}$ to $\frac{0, 0, 1, 2, 1}{0, 0, 0, 2, 1}$; of the eighth, $\frac{0, 0, 1, 2, 1}{0, 0, 0, 1, 1}$ to $\frac{0, 0, 1, 2, 1}{0, 0, 1, 2, 1}$; of the ninth, $\frac{0, 0, 1, 2, 1}{0, 0, 0, 2, 1}$ to $\frac{0, 0, 1, 2, 1}{0, 0, 1, 2, 1}$; of the tenth, $\frac{0, 0, 1, 1, 1}{0, 0, 1, 2, 1}$ to $\frac{0, 0, 1, 1, 2}{0, 0, 1, 2, 1}$; of the twelfth, $\frac{0, 0, 1, 1, 0}{0, 0, 1, 2, 1}$ to $\frac{0, 0, 2, 1, 0}{0, 0, 2, 2, 1}$; of the thirteenth, $\frac{0, 0, 2, 1, 0}{0, 0, 1, 2, 1}$, $\frac{0, 0, 2, 1, 0}{0, 0, 2, 2, 1}$, $\frac{1, 0, 2, 1, 0}{0, 1, 2, 2, 1}$ or $\frac{1, 0, 2, 1, 1}{0, 1, 3, 2, 1}$; of the penult, $\frac{1, 0, 2, 1, 0}{0, 1, 3, 2, 1}$; of the anal, $\frac{1, 0, 2, 0, 0}{0, 1, 3, 1, 0}$. Anal coxae armed laterally as in the adult.

Claw of female gonopods very small and weak, consisting of a single pointed process or with the lateral divisions indicated as slender

spinous points at base of the median process. Either two or three articles present. Basal spines 1+1; slender and acutely acuminate from base distad.

Length 4.75 to 5.75 mm.

Agentialis I.—Body very pale, suffused with violaceous. Head, prehensorial feet and prosternum light orange. Antennae dilute yellow. Legs almost without pigment excepting the caudal pairs which are yellow.

Antennae composed of seventeen articles. Distally submoniliform. Bristles long, subsparse.

Ocelli four in two series:—thus, 1+2, 1. The single ocellus smaller than the caudal one of the upper series; the two ocelli of upper series well separated.

Prosternal teeth and spines of nearly same form and relations as in the adult.

Coxal pores 1, 1, 1, 1.

Anal glands still evident.

Length cir. 4 mm.

TYPE LOCALITY.—New Jersey (Masonville!).

LOCALITIES.—New Jersey (Masonville! Camden! Westfield!); Pennsylvania (Upsal! Philadelphia!).

In New Jersey this species is found occurring commonly under the bark of fallen trees in pine woods.

This form is evidently very close to *monticolens* with which the first inclination was to unite it; but the examination of a considerable number of specimens from the localities above listed showed no variation in a number of differences presented. The most definite and readily usable difference is in the spining of the eleventh and twelfth pairs of legs, the formula for the ventral surface of which in *monticolens* is 0, 0, 3, 3, 2 whereas in all specimens of *opicolens* examined it is uniformly 0, 0, 2, 3, 2. A larger number of the posterior coxae are dorsally armed, but this in other species is subject to considerably variation so that it may not prove of distinctive value in the present case. *Garibius opicolens* is more robust and also presents well marked differences in color and color-pattern from that shown by the individuals of *monticolens* thus far observed.

GARIBIUS DENDROPHILUS, sp. nov.

DIAGNOSIS.—Dorsal spines of anal legs, 1, 0, 2, 0, 0; of the penult, 1, 0, 2, 1, 0. Ventral spines of the thirteenth legs, 0, 0, 3, 3, 2. Dorsal

spines of the twelfth legs, 1, 0, 2, 1, 1; ventral, 0, 0, 2, 3, 2. Dorsal spines of the eleventh legs, 0, 0, 2, 1, 2; ventral, 0, 0, 1, 3, 2. Dorsal spines of the first legs, 0, 0, 2, 2, 1; ventral 0, 0, 1, 3, 2. None of the coxae laterally armed.

Ocelli mostly near twelve, in three series.

Prosternum cir. 1.46 times wider than long.

Penult legs of male without lobes and the crest of tibia of anal legs almost obsolete.

Head subcordate; wider than long.

Robust, in type not fully seven times longer than width of tenth plate.

Dorsum reddish brown, with caudal margins of plates darkened; head light ferruginous, darker along caudal portion.

DESCRIPTION.—Dorsum reddish brown, somewhat darker along caudal borders of plates; last two plates brighter, more reddish in color. Head light ferruginous, darker across caudal portion. Prosternum yellow ferruginous, the prehensors being yellow. Antennae yellow proximally and more or less violaceous brown elsewhere excepting at very tip which is again lighter. Venter light brown of violaceous tinge; the anterior plates somewhat darker, more reddish, and the posterior ones decidedly darker, subferruginous. Legs pale; the anal and penult pairs bright yellow excepting at bases.

Body considerably more robust than in *G. monticolens* and *opicolens*, in the type being not fully seven times as long as width of tenth dorsal plate.

Head subcordate. Widest at or a little in front of marginal breaks between which and the eyes the sides are convex; sides converging from breaks caudad about the well rounded corners to the caudal margin which is straight or slightly incurved. Anterior margin between eyes substraight, crenately indented a little each side of median line. Hairs of moderate length; sparse. Wider than long about in ratio 58:55.

Antennae rather short; 2.75— times longer than the head. Composed of the usual twenty articles which decrease uniformly in diameter and length distad; rather loosely joined; ultimate article relatively shorter than in *monticolens* and *opicolens*. Hairs long and straight, of moderate denseness.

Eyes consisting mostly of about twelve ocelli arranged in three series:—thus, 1+5, 4, 2; 1+5, 3, 2. Single ocellus largest.

Prosternum cir. 1.46 times wider than long. Distance between chitinous spots nearly three times (in type 2.9—) as great as width at

level of bottom of median incision; 3.84 times the length of the dental line. Mesal incision v-shaped, subacute at bottom but with the converging sides a little concave. Line of bases of teeth distinctly recurved; but the inner tooth on each side larger than the outer so that the line of apices is nearly straight. Teeth acute, the interval between those of each pair angularly rounded at bottom. Ectal spine stouter at base than the bristles, rapidly acuminate, short.

Coxal pores moderately small; in type, 2, 3, 3, 2.

The single article of the gonopod of male broad but short and only slightly exposed. Broadly truncate. A moderately long median apical bristle and at least one more ectal in position.

Spines of first to eighth pairs of legs, $\frac{0, 0, 2, 2, 1}{0, 0, 1, 3, 2}$; of ninth and tenth pairs, $\frac{0, 0, 2, 2, 2}{0, 0, 1, 3, 2}$; of the eleventh, $\frac{0, 0, 2, 1, 2}{0, 0, 1, 3, 2}$; of the twelfth, $\frac{1, 0, 2, 1, 1}{0, 0, 2, 3, 2}$; of the thirteenth, $\frac{1, 0, 2, 1, 1}{0, 0, 3, 3, 2}$; of the penult, $\frac{1, 0, 2, 1, 0}{0, 1, 3, 3, 1}$; of the anal, $\frac{1, 0, 2, 0, 0}{0, 1, 3, 2, 0}$ claw one. None of the coxae laterally armed.

Length cir. 7.5 mm. Length of antennae 2.7 mm.; of anal legs, 3+ mm.

LOCALITY.—Pennsylvania (Philadelphia!).

One male thus far known. Like the preceding species, the type of this one was found under the bark of a fallen log.

This species appears to be related somewhat closely to the two preceding ones; but it is very readily distinguishable through lacking a lateral spine on the anal coxae; the ventral spines of the anal leg being 0, 1, 3, 2, 0 instead of the more usual 0, 1, 3, 1, 0; the absence of spine from trochanter of thirteenth legs; the dorsal spines of the eleventh legs being 0, 0, 2, 1, 2 instead of 0, 0, 2, 1, 1 or 1, 0, 2, 1, 1, and the ventral 0, 0, 1, 3, 2 instead of 0, 0, 2, 3, 2 or 0, 0, 3, 3, 2; the ventral spines of the first legs being 0, 0, 1, 3, 2 instead of 0, 0, 1, 2, 1 or 0, 0, 1, 3, 1; through the almost obsolete crest of tibia of anal legs; and finally through apparently greater robustness.

GARIBIUS PAGOKETES, sp. nov.

DIAGNOSIS.—Dorsal spines of anal legs 1, 0, 2, 0, 0. Dorsal spines of penult legs, 1, 0, 2, 1, 0. Ventral spines of thirteenth legs, 0, 1, 3, 2, 2. Dorsal spines of twelfth legs 0, 0, 2, 1, 1; ventral 0, 0, 2, 3, 2. Dorsal spines of eleventh legs 0, 0, 2, 1, 2; ventral, 0, 0, 2, 3, 2. Dorsal spines of first legs 0, 0, 2, 1, 1; ventral, 0, 0, 1, 2, 1. All legs with the

third joint armed both dorsally and ventrally. Last two pairs of coxae laterally armed.

Antennae of usual structure and relations.

Ocelli 10 in two or three series.

Prosternum 1.65— times wider than long.

Length (type) 8 mm.

Body eight times longer than width of tenth plate.

Head equal in length and breadth.

First dorsal plate 1.65 times wider than long.

Dorsum brown, darker caudad. Head light in front of suture.

Antennae rufous yellow distad.

DESCRIPTION.— Dorsum brown, the caudal plates becoming somewhat dilute chestnut. Head pale in front of the suture. Antennae brownish, distally lighter, yellow of somewhat rufous tinge. Legs brownish yellow, the anterior pairs lightest, caudal pair more yellow distally. Venter light brown, darker, more reddish, caudad. Prosternum testaceous.

Head subcordate; equal in length and breadth; of nearly uniform width between eyes and lateral breaks or a little widest at the latter; widely rounded from the breaks caudad about the corners.

Antennae short. Articles distad of the third short and very short, the distal ones proportionately somewhat longer than the more proximal.

Eyes in type consisting of ten ocelli arranged in two or three series:— thus, 1+5, 4 to 1+4, 3, 2. Single ocellus large.

Prosternum about 1.65— times wider than long. Distance between chitinous spots 3.75 times width at median incision, 4 times the dental lines.

First dorsal plate 1.65 times wider than long. Posterior angles of eleventh and thirteenth dorsal plates obliquely excised.

Coxal pores small, circular, decreasing proximad; in number, 3, 3, 3, 3.

Spines of first legs, $\frac{0, 0, 2, 1, 1}{0, 0, 1, 2, 1}$; of second to sixth, $\frac{0, 0, 2, 2, 1}{0, 0, 1, 3, 1}$; of seventh to ninth, $\frac{0, 0, 2, 2, 2}{0, 0, 1, 3, 2}$; of tenth, $\frac{0, 0, 2, 2, 2}{0, 0, 2, 3, 2}$; of eleventh, $\frac{0, 0, 2, 1, 2}{0, 0, 2, 3, 2}$; of the twelfth, $\frac{0, 0, 2, 1, 1}{0, 0, 2, 3, 2}$; of the thirteenth, $\frac{1, 0, 2, 1, 1}{0, 1, 3, 2, 2}$; of the penult, $\frac{1, 0, 2, 1, 0}{0, 1, 3, 2, 1}$, claws 3; of the last, $\frac{1, 0, 2, 0, 0}{0, 1, 3, 1, 0}$, claw single.

Claw of female gonopods proportionately wide, tripartite, the divisions not differing much in length, acute. Inner spine of each pair markedly shorter than the outer (*maturus*). Spines not acuminate

from base, but somewhat constricted near middle of length and of greatest width immediately below the flattened acuminate apical portion, which is short.

In the type (♀) the widths of head, and first, third, eighth, tenth, and twelfth dorsal plates are as 50:46:48:56:57:56.

Length of type 8 mm.; width of tenth plate 1 mm.; length of antennae 2.4 mm.; of anal legs about 3 mm.

TYPE LOCALITY.—Massachusetts (Blue Hills!).

The closest allies of this species would seem to be *G. monticolens*, *opicolens*, and *dendrophilus*, from which it is to be separated by the characters assigned in the key and the diagnosis. The male is not known.

GARIBIUS MISSISSIPPIENSIS, sp. nov.

DIAGNOSIS.—Dorsal spines of anal legs 1, 0, 2, 0, 0. Dorsal spines of penult legs 1, 0, 2, 1, 0. Ventral spines of thirteenth legs 1, 3, 3, 2. Dorsal spines of twelfth legs 1, 0, 2, 1, 1; ventral, 0, 0, 2, 3, 2. Dorsal spines of eleventh legs 1, 0, 2, 1, 2; ventral, 0, 0, 2, 3, 2. Dorsal spines of first legs, 0, 0, 1, 1, 1; ventral, 0, 0, 0, 2, 1. All legs with the third joint dorsally armed, and all excepting the first with this joint also ventrally armed. Last two pairs of coxae laterally armed.

Ocelli about 12, in three series.

Antennae as usual.

Prosternum $1\frac{3}{4}$ times wider than long. Distance between chitinous spots 2.5 times width at median incision; $4\frac{1}{2}$ times the dental line. Incision strictly v-shaped.

Body 7.5 times longer than width of tenth plate.

Length (type), 6.75 mm.

Head cordate; clearly wider than long.

First dorsal plate $1.8+$ times wider than long.

Fifth article of anal leg of male raised into an edge along dorso-mesal side with the ridge at caudal end elevated into a crest bearing a number of hairs of the usual character. Penult legs without lobes.

Pale testaceous with the posterior plates orange. Head orange or light ferruginous.

DESCRIPTION.—Dorsum pale testaceous with the posterior plates orange. Head orange or light ferruginous. Antennae yellow. Prosternum orange of paler shade than head. Venter pale with caudal plates more or less orange. Legs pale excepting the posterior pairs which are bright orange.

Body more robust relatively than in related species; 7.5 times longer than width of tenth dorsal plate. Widths of head and of first, third, eighth, tenth, and twelfth plates to each other as 43:37:38:46:47:46.

Head cordate. Widest a little back of eyes from where the sides gently converge caudad; rather strongly narrowed cephalad. Wider than long in about ratio 43:40. Hairs rather short, sparse.

Antennae short, reaching the sixth segment or nearly so. Composed of the usual twenty articles which decrease in size distad from the second, and, excepting those at the two ends, are very short. Hairs short and not very dense.

Eyes composed mostly of about 12 ocelli which are arranged in three series:—thus, 1+5, 4, 2; 1+4, 4, 3. Single ocellus small, not larger than others. Seriate ocelli not contiguous, the caudal ones not larger than the anterior; all small.

Prosternum $1\frac{1}{2}$ times wider than long. Distance between chitinous spots 2.5 times width at level of bottom of median incision; $4\frac{1}{2}$ times the dental line. Median incision strictly v-shaped, being angular at bottom with sides straight or but very slightly concave. Teeth small and acute; the inner slightly larger than the outer; line of apices weakly recurved or straight. Spine inserted a little ectad of outer tooth as usual; slender, straight.

First dorsal plate widest at or near anterior end, the sides at first gently and then more strongly converging caudad; 1.8+ times wider than long. Caudal corners of ninth, eleventh, and thirteenth dorsal plates straight or a little rounded, none of these either at all produced or excised.

Coxal pores very small: 2, 3, 3, 2-3, 3, 3, 3.

Spines of first legs, $\frac{0, 0, 1, 1, 1}{0, 0, 0, 2, 1}$; of the second to seventh inclusive, $\frac{0, 0, 2, 2, 1}{0, 0, 1, 2, 1}$; of the eighth, $\frac{0, 0, 2, 2, 1}{0, 0, 1, 3, 2}$; of the ninth, $\frac{0, 0, 2, 2, 1}{0, 0, 2, 3, 2}$; of the tenth, $\frac{0, 0, 2, 2, 2}{0, 0, 2, 3, 2}$; of the eleventh, $\frac{1, 0, 2, 1, 2}{0, 0, 2, 3, 2}$; of the twelfth, $\frac{1, 0, 2, 1, 1}{0, 0, 2, 3, 2}$; of the thirteenth, $\frac{1, 0, 2, 1, 1}{0, 1, 3, 3, 2}$; of the penult, $\frac{1, 0, 2, 1, 0}{0, 1, 3, 2, 1}$, with three claws; of the anal, $\frac{1, 0, 2, 0, 0}{0, 1, 3, 1, 0}$, claw single. Last two pairs of coxae laterally armed.

Anal legs of male short and moderately inflated; fifth article narrowed to an edge along dorso-mesal line with at caudal end of this ridge a low, dorsally gently convex, crest bearing a number of hairs of the usual character; longitudinally sulcate laterally. Penult legs less inflated; without lobes.

Length of type 6.75 mm.; width of tenth plate, .9 mm.; length of antenna, 2.25 mm.; of anal leg, 2.5 mm.

LOCALITY.—Mississippi (Brookhaven!).

GARIBIUS GEORGIAE, sp. nov.

DIAGNOSIS.—Dorsal spines of anal legs, 1, 0, 2, 0, 0. Dorsal spines of penult legs, 1, 0, 2, 1, 0. Ventral spines of thirteenth legs, 0, 0, 2, 3, 2. Dorsal spines of twelfth legs, 0, 0, 2, 1, 1 - 1, 0, 2, 1, 1; ventral, 0, 0, 2, 3, 2. Dorsal spines of eleventh legs, 0, 0, 2, 1, 1; ventral, 0, 0, 2, 2, 2. Dorsal spines of first legs, 0, 0, 1, 1, 1; ventral, 0, 0, 0, 2, 1. Third joint of all legs dorsally armed; but about first eight or nine pairs with this joint unarmed ventrally. None of the coxae laterally armed.

Antennae as usual.

Ocelli about 9 to 11 in three series.

Prosternum 1.9 times wider than long. Distance between chitinous spots 2.44 times width at median incision; 3.44 times the dental line.

Coxal pores 2, 3, 3, 2 - 2, 3, 3, 3.

Claw of gonopods of the female small; tripartite; lobes acute with the median considerably longest. Outer spine longer than the inner; moderately long and slender, attenuated from the base distad, the sides more strongly rounding in toward the apex.

Anal leg of male modified as usual, the fifth article bearing above a low ridge elevated into a low crest at caudal end.

Length 5.5 to 7.5 mm.

Body 9 times as long as width of tenth plate.

Head equal in length and breadth.

First dorsal plate 1.84 times wider than long.

Dorsum yellow, with caudal plates verging to light orange. Head light orange.

DESCRIPTION.—Yellow above, with the caudal plates tending toward light orange. Head light orange. Antennae yellow. Prosternum paler than head, being more of a lemon yellow. Venter very pale excepting the caudal plates which are like the prosternum. Legs pale, almost whitish, excepting the caudal pairs which are orange brown.

Rather slender, being nine times as long as the width of the tenth plate. Widths of head and of first, third, eighth, tenth, and twelfth plates to each other as 40: 35: 36: 42: 43: 40.

Head equal in length and breadth or in the male it may be a little longer. Widest a little caudad of eyes from where the sides moderately converge caudad; rather strongly narrowed cephalad. Hairs short and sparse.

Antennae short; moderately attenuated; composed of twenty articles, most of which are short and very short, the ultimate relatively long. Hairs of moderate length, not very dense.

Eyes each composed of from 9 to 11 ocelli arranged in three series:—thus, 1+4, 4, 2; 1+4, 3, 3; 1+4, 3, 1; 1+4, 3, 2. Single ocellus of same size as or a little smaller than those of the upper series, the seriate ocelli decreasing in size cephalad and ventrad. All ocelli pale.

Prosternum 1.9+ times wider than long. Distance between chitinous spots 2.44 times the width at level of bottom of median incision; 3.44+ times the length of the dental line. Median incision rather narrowly v-shaped, the sides straight. Teeth nearly equal or with inner on each side somewhat larger making the line of apices straight or nearly so; acute, with sides strictly straight and the interval between the two of each pair angular, not at all rounded. Spine fine, but slightly stouter than the hairs; straight; inserted a little ectad of base of outer tooth. Lateral edges of anterior portion gently convex cephalad and bending ectad caudally as usual.

First dorsal plate with sides almost parallel from caudal third cephalad; 1.84 times wider than long. Posterior corners of the ninth, eleventh, and thirteenth plates rounded or rather obliquely excised.

Coxal pores very small; 2, 3, 3, 2—2, 3, 3, 3.

Spines of first legs, $\frac{0, 0, 1, 1, 1}{0, 0, 0, 2, 1}$; of the second and third, $\frac{0, 0, 1, 2, 1}{0, 0, 0, 2, 1}$; of the fourth to sixth, $\frac{0, 0, 1, 2, 1}{0, 0, 0, 2, 1}$ or $\frac{0, 0, 2, 2, 1}{0, 0, 0, 2, 1}$; of the seventh, $\frac{0, 0, 2, 2, 1}{0, 0, 0, 2, 1}$ or $\frac{0, 0, 2, 2, 1}{0, 0, 0, 2, 2}$; of the eighth, $\frac{0, 0, 2, 2, 2}{0, 0, 0, 2, 1}$ or $\frac{0, 0, 2, 2, 2}{0, 0, 0, 2, 2}$; of the ninth, $\frac{0, 0, 2, 2, 2}{0, 0, 0, 2, 2}$ or $\frac{0, 0, 2, 2, 2}{0, 0, 1, 2, 2}$; of the tenth, $\frac{0, 0, 2, 2, 2}{0, 0, 1(0), 2, 2}$; of the eleventh, $\frac{0, 0, 2, 1, 1}{0, 0, 2, 2, 2}$; of the twelfth, $\frac{0, 0, 2, 1, 1}{0, 0, 2, 3, 2}$ or $\frac{1, 0, 2, 1, 1}{0, 0, 2, 3, 2}$; of the thirteenth, $\frac{1, 0, 2, 1, 1}{0, 0, 2, 3, 2}$; of the penult, $\frac{1, 0, 2, 1, 0}{0, 1, 3, 2, 1}$; claws 3; of the anal, $\frac{1, 0, 2, 0, 0}{0, 1, 3, 1, 0}$ the claw single. None of the coxae laterally armed.

Anal legs of male short and moderately inflated; longitudinally sulcate laterally but not dorsally; fifth joint complanate above, extended in a low, rather sharply edged, longitudinal ridge along dorso-mesal side which ends at caudal end in the usual crest, this being low with its free edge a little convex and bearing a series of hairs which are

curved at distal ends. Penult legs without any special modifications; moderately and uniformly inflated.

Claw of female gonopods small; considerably curved; tripartite; lobes acute with the median considerably longest. Outer of basal spines larger than the inner; moderately long and slender, a little attenuated from base distad, the sides toward end more strongly rounding in to the apex. Inner side of first article straight, a little diverging from its mate at anterior end.

Length of types from 5.5 to 7.5 mm. A specimen 7.3 mm. long has tenth plate .82 mm. wide, antennae 2.4 mm. long, and anal leg about 2.2 mm. long.

LOCALITY.—Georgia (Atlanta!).

GARIBIUS ALABAMAE, sp. nov.

DIAGNOSIS.—Dorsal spines of anal legs, 1, 0, 2, 0, 0. Dorsal spines of penult legs 1, 0, 2, 1, 1. Ventral spines of thirteenth legs, 0, 0, 2, 3, 2. Dorsal spines of twelfth legs, 0, 0, 2, 2, 2; ventral, 0, 0, 1, 2, 1. Dorsal spines of eleventh legs, 0, 0, 0, 2, 2; ventral, 0, 0, 0, 2, 1. Dorsal spines of first legs 0, 0, 0, 1, 1; ventral, 0, 0, 0, 1, 1. Third joint of first eleven pairs of legs or thereabouts unarmed either dorsally or ventrally. Last pair of coxae laterally armed.

Antennae as usual.

Ocelli about 6 in two series.

Prosternum 1.53 times wider than long. Distance between chitinous spots 2.4+ times width at median incision; 3.42 times dental line.

Crest of anal legs of male broader and more rounded than in related species; bearing numerous hairs of the usual type.

Length of type 5 mm.

Body 7.6+ times longer than the width of the tenth plate.

Head considerably longer than wide.

Dorsum anteriorly greyish yellow, becoming clearer yellow and light orange caudad. Head light orange.

DESCRIPTION.—Above the body is greyish yellow, becoming clearer yellow and light orange caudad. Head light orange. Antennae yellowish. Prosternum pale orange or yellow. Venter dilute yellow, deeper yellow caudad. Legs very pale excepting the caudal ones which are bright yellow.

Length about 7.6+ times the width of tenth dorsal plate. Widths of head and of first, third, eighth, tenth, and twelfth dorsal plates to

each other as 31: 31+ : 31: 34: 34: 33, the body being thus not strongly attenuated.

Head elongate, being considerably longer than wide (34:31). Hairs of moderate length, sparse.

Antennae short; moderately attenuated. Articles mostly very short, decreasing distad as usual; the ultimate moderately long. Hairs as usual in the genus.

Eyes composed of about 6 ocelli in two series:— thus, 1+3, 2. Single ocellus about equal in size to, or a little smaller than, the first one of the upper series. Seriate ocelli decreasing cephalad and ventrad; all pale.

Prosternum 1.53 times wider than long. Distance between chitinous spots 2.4+ times width at level of bottom of mesal incision; 3.42 times as great as the dental line. Teeth small and acute; interval between each pair rounded at bottom; inner tooth somewhat larger than the outer and the line of apices straight. Mesal incision rather wide and shallow, sides concave but meeting at an acute angle at bottom.

First dorsal plate widest a little back of the anterior end, the sides from there caudad at first slightly and then rather strongly converging. Posterior corners of ninth, eleventh, and thirteenth dorsal plates all straight on caudal side or a little rounded.

Spines of first legs, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 1(0), 1}$; of the second, $\frac{0, 0, 0, 1(2), 2}{0, 0, 0, 2(1), 1}$; of the third to eleventh inclusive, $\frac{0, 0, 0, 2, 2}{0, 0, 0, 2, 1}$; of the twelfth, $\frac{0, 0, 2, 2, 2}{0, 0, 1, 2, 1}$; of the thirteenth, $\frac{1, (0), 2, 1, 1}{0, 0, 2, 3, 2}$; of the penult, $\frac{1, 0, 2, 1, 1}{0, 1, 3, 2, 1}$, claws 2 (3); of the anal, $\frac{1, 0, 2, 0, 0}{0, 1, 3, 1, 0}$, claw single. Last pair of coxae laterally armed.

Gonopods of male well exposed, convexly rounded, wart-like; bearing a single moderately long bristle.

Anal legs of male short, considerably inflated; fifth joint bearing at its distal end a low eminence which is relatively broader and more rounded than in related species, this lobe bearing over its surface rather numerous hairs of the usual character. Penult legs also inflated, but without any special lobes.

Length of type 5 mm.; width of tenth plate .65 mm.; length of antenna 1.6 mm.; anal leg of same length as antenna or nearly so.

LOCALITY.— Alabama (Morgan!).

A single male was secured.

GARIBIUS BRANNERI (Bollman).

Lithobius branneri Bollman, Ann. N. Y. acad. sci., 1888, 4, p. 107.

DESCRIPTION.— The original description, rearranged, is as follows:—
Light chestnut-brown or orange; head and antennae scarcely darker; feet orange.

Slender, smooth, very sparsely pilose.

Head rounded triangular, narrowest before.

Antennae short; joints 20, short.

Ocelli 6–8, arranged in four or five rows.

Prosternal teeth 4, small.

[Posterior angles of none of the dorsal plates produced].

Coxal pores 2, 3, 3, 2–3, 4, 4, 3, small and round.

First pair of feet armed with 0, 2, 1 spines; penultimate with 1, 3, 1, 1–1, 3, 2, 1; last with 1, 3, 1, 0.

In the male the fifth joint of the anal leg is produced into a short pilose lobe, and is depressed.

Claw of the female genitalia short, wide, bi- or tri-partite; spines slender, subequal, outer strongly toothed.

Length of body 5–10 mm.

TYPE LOCALITY.— Tennessee (Knoxville!).

LOCALITIES.— Tennessee (Knoxville! Beaver Creek; Mossy Creek).

TIDABIUS, gen. nov.

Antennae short; composed of from 25 to 35 articles, but of mostly near 32.

Eyes composed mostly of from 9 to 14 ocelli which are arranged usually in three series; single ocellus largest.

Prosternum from 1.55 to 1.65 times wider than long, never less. Teeth 2+2; line of apices somewhat recurved. Spine bristle-like. Median incision v-shaped, being acute at bottom, or very rarely more or less rounded.

Body always considerably attenuated from the tenth plate cephalad with the first plate always and mostly considerably narrower than the third.

Posterior angles of none of the dorsal plates produced; or, often, with thirteenth or with eleventh and thirteenth showing a very weak production of these angles.

Coxal pores small and circular, uniseriate.

Gonopods of female tripartite with lobes short, the lateral ones equal and with their apices usually in nearly same transverse line. Basal spines 2+2 or, rarely, 3+3; proximal portion of spine relatively broad with sides parallel or diverging to where the acutely acuminate terminal portion begins more or less abruptly (Plate 4, fig. 5).

Posterior coxae always wholly unarmed. Anal legs with the dorsal spines uniformly 0, 0, 2, 0, 0; ventral spines, 0, 1, 3, 1, 0 or, in some, 0, 1, 3, 2, 0; claws always 2 or 3. Penult legs with dorsal spines 0, 0, 3, 1, 0, or, rarely, 0, 0, 3, 1, 1; ventral spines, 0, 1, 3, 3, 1, to 0, 1, 2, 3, 1; claws 2 or 3. Third joint of first 7 to 9 pairs of legs mostly unarmed, rarely armed. Tibia of none of the legs with more than one ventral spine. All tarsi biarticulate.

Posterior legs in both sexes short and moderately inflated, longitudinally more or less sulcate dorsally; in male presenting no special modifications, or with legs but little more crassate.

Length between 5.5 and 10 mm.

TYPE.—*T. tivius* Chamberlin.

DISTRIBUTION.—The known species are distributed across the continent through the Northern United States from Washington and Oregon to the Atlantic and from there into the Southeastern States.

These small and wide-spread lithobiids never exceed 10 mm. in length. In most of the species the head varies from yellowish to ferruginous and is distinctly lighter than the dorsum. The venter commonly is suffused anteriorly with violaceous. The posterior legs are typically more brightly colored distad. These forms occur commonly under leaves and sticks of willows and other trees and shrubs along streams, under bark of logs, among stones at the water's edge and in other similar places. Where found they usually occur in considerable numbers. The species to one examining them at first commonly seem very close in general structure and appearance and are to be discriminated only upon careful study and comparison. The following key will aid in separating average individuals.

Key to species of Tidabius.

- a. Ventral spines of anal legs 0, 1, 3, 2, 0 or 0, 1, 3, 3, 0.
 - b. Third article of all legs, or of all excepting the first two pairs, ventrally armed. *T. poaphilus*, sp. nov.
 - bb. Third article of at least the first five pairs of legs unarmed ventrally.

- c. Dorsal spines of penult legs 0, 0, 3, 1, 1.
T. anderis, sp. nov.
- cc. Dorsal spines of penult legs 0, 0, 3, 1, 0.
 - d. Anal leg with 3 distinct claws; basal spines of ♀ gonopods 3+3.
T. bonvillensis Chamberlin.
 - dd. Anal leg with but 2 distinct claws; basal spines of ♀ gonopods 2+2.
 - e. Body 8 times, or but little more, as long as width of tenth plate.
T. suitus, Chamberlin.
 - ee. Body 9 or more times as long as width of tenth plate.
T. nasintus, sp. nov.
- aa. Ventral spines of anal legs 0, 1, 3, 1, 0.
 - b. Length of body of adults less than 8 mm.; mostly from 7.25 to 8.25 times longer than width of tenth plate.
T. tivius Chamberlin.
 - bb. Length of body of adults 8 mm. or more; 8.5 or more times longer than width of tenth plate.
 - c. Ventral spines of eleventh legs 0, 0, 1, 3, 1; of the thirteenth, 0, 1, 2, 3, 1 or 0, 0, 2, 3, 1.
Body 9 to 9.5 times longer than width 10th plate.
T. opiphilus, sp. nov.
 - cc. Ventral spines of eleventh legs 0, 0, 2, 3, 1; of the thirteenth, 0, 1, 3, 3, 1.
 - d. Dorsum brown; head brown of ferruginous caste; body about 8.5 times longer than width of 10th plate.
T. pallidus alabamensis, subsp. nov.
 - dd. Dorsum yellow; head light orange; body about 9 times longer than width of 10th plate. *T. pallidus*, sp. nov.

TIDABIUS NASINTUS, sp. nov.

DIAGNOSIS.— Ventral spines of anal legs 0, 1, 3, 2, 0. Dorsal spines of penult legs, 0, 0, 3, 1, 0. Third joint of eleventh to fourteenth legs with three dorsal spines. Ventral spines of tenth and eleventh legs 0, 0, 1, 2, 1. Ventral spines of first legs, 0, 0, 0, 1, 1.

Articles of antennae (in type) 30.

Ocelli 11 in three series.

Distance between chitinous spots of prosternum 2.58 times width at median incision; 3.75–4 times the dental line.

Head slightly wider than long.

Coxal pores 2, 3, 3, 3.

Slender, about 9.3 times longer than the width of tenth plate.

First dorsal plate only $1\frac{2}{3}$ times wider than long.

Outer of basal spines on each side longer than the inner with which it stands at an angle; spines broad at base with sides slightly diverging to middle where acuminate portion begins.

Dorsum light brown, the caudal plates darker. Head darker brown or dilute chestnut with frontal region paler.

DESCRIPTION.—Above light brown with the caudal plates darker. Head darker brown or dilute chestnut, the frontal region paler. Antennae brown proximally, light at tips. Prosternum brown; prehensorial feet yellowish. Venter pale with the caudal plates darker, brownish. Legs like the corresponding plates of venter, the posterior pairs there being darker, brownish, but brighter distad as usual.

Body slender, being in the type 9.3 times longer than the width of the tenth plate. The widths of head and of the first, third, eighth, tenth, and twelfth plates to each other approximately as 41:35:38:43:45:43.

Head subcordate. Slightly wider than long (ratio about 41:40). Not much varying in width between caudal edge of eyes and the marginal breaks.

Antennae short. Composed of thirty articles which distad of the second or third become very short and closely arranged. Hairs short extending in part distoectad and in part proximoectad as usual.

Eye, in the type, composed of eleven ocelli arranged in three series:—thus, 1+4, 3, 3. Single ocellus largest. Ocelli of upper series larger and paler than others which decrease in size ventrad.

Prosternum, in type, 1.58— times wider than long. Distance between chitinous spots 2.58 times width at level of bottom of median incision; 3.87 times the dental line. Median incision rather small, v-shaped. Teeth small, acute, with sides nearly straight; inner tooth a little larger than the outer but the line of apices clearly recurved. Distance from tip to tip of mesal teeth about equal to or a little less than that from tip to tip of the two of each lateral pairs. Lateral margin of anterior portion convex anteriorly and concave posteriorly, where it bends ectad at end as usual.

First dorsal plate $1\frac{2}{3}$ times wider than long; widest a little back of the anterior end where the sides are convex, the sides then moderately converging caudad to the rounded corners. Caudal margin of the ninth plate straight or nearly so; angles of eleventh and thirteenth very slightly produced, the process rounded; posterior corners of other minor plates more or less obliquely excised.

Coxal pores circular; small, decreasing in size proximad on each coxa; 2, 3, 3, 3.

Spines of first legs, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 1, 1}$; of the second, $\frac{0, 0, 1, 1(2), 1}{0, 0, 0, 2, 1}$; of the third and fourth, $\frac{0, 0, 1, 2, 1}{0, 0, 0, 2, 1}$; of the fifth, $\frac{0, 0, 2, 2, 1}{0, 0, 0, 2, 1}$; of the seventh and eighth, $\frac{0, 0, 2, 2, 2}{0, 0, 0, 2, 1}$; of the ninth, $\frac{0, 0, 2, 2, 2}{0, 0, 0, 2, 1}$ or $\frac{0, 0, 2, 2, 2}{0, 0, 1, 2, 1}$; of the tenth, $\frac{0, 0, 2, 2, 2}{0, 0, 1, 2, 1}$; of the eleventh, $\frac{0, 0, 3, 2, 2}{0, 0, 1, 2, 1}$; of the twelfth, $\frac{0, 0, 3, 1, 1}{0, 0, 2, 2, 1}$; of the thirteenth, $\frac{0, 0, 3, 1, 1}{0, 1, 2, 3, 1}$; of the fourteenth, $\frac{0, 0, 3, 1, 0}{0, 1, 3, 3, 1}$; of the anal, $\frac{0, 0, 2, 0, 0}{0, 1, 3, 2, 0}$, with two claws. None of the coxae at all armed.

Anal and penult legs of the female short, weakly inflated; especially the fourth joint longitudinally sulcate above.

Claw of female gonopods short and relatively wide; tripartite; the lobes short but distinct, the median longer than the lateral which are equal in size and at the same level. Outer of the two basal spines of each pair with which its wider surface stands at an angle as usual; spines broad at base with sides slightly diverging to the middle and then converging to a very acute apex, the sides of the acuminate portion being somewhat incurved. Inner side of first article of gonopods slightly curving ectad at proximal end and then a little diverging from its fellow.

Length of type 8 mm.; width of tenth plate, .86 mm.; length of antennae, 2.3 mm.; of anal leg, 2.1 mm.

LOCALITY.—Mississippi (Jackson!).

TIDABIUS SUITUS (Chamberlin).

Lithobius cantabrigensis var. *suitus* Chamberlin, Ann. Ent. soc. America, 1911, 4, p. 41.

DIAGNOSIS.—Ventral spines of anal legs, 0, 1, 3, 2, 0. Dorsal spines of penult legs 0, 0, 3, 1, 0 or 0, 0, 2, 1, 0. Third joint of tenth to fourteenth legs inclusive armed with three dorsal spines. Ventral spines of tenth and eleventh legs, 0, 0, 1, 2, 1. Ventral spines of first legs, 0, 0, 0, 1, 1 or 0, 0, 0, 2, 1. None of the coxae armed.

Articles of antennae 29–32.

Ocelli 8–11 in 2 or 3 series.

Distance between chitinous spots of prosternum 2–2.25 times width at median incision; 3.75–4 times as great as the dental line. Median incision rather large, widely rounded at bottom.

Coxal pores 2, 3, 3, 2-3, 4, 4, 3.

Head subcordate, wider than long.

Body moderate; about eight times longer than the width of the tenth plate.

Median lobe of claw of female gonopods but little longer than the lateral. Basal spines very broad proximally, sides parallel or slightly diverging to about the middle where the acuminate portion begins.

Dorsum brown, with posterior segments darker and more reddish as are also sometimes the first ones. Head brown of dilute ferruginous tinge, especially anteriorly.

DESCRIPTION.—Brown above, with the posterior segments considerably darker and more reddish; some of the anterior plates may also be darker, especially along the caudal borders, these also often showing a violaceous tinge. Head brown of dilute ferruginous tinge, especially anteriorly. Antennae brown of purplish tinge, distally lighter, yellow or yellowish of dilute ferruginous tinge. Prosternum brown, the prehensorial feet lighter. Venter very pale, the anterior plates showing a distinct violaceous or purplish tinge and the caudal plate much deeper in color and more or less of reddish tinge. Legs like the corresponding plates of venter; the posterior pairs darker brown with the distal joints light.

Body moderate, being on an average 8+ times longer than wide. Widths of head and of first, third, eighth, tenth, and twelfth plates to each other as 25.5: 22: 22: 26: 27: 26.

Head subcordate; wider than long in about ratio 25.5: 24. Hairs short, sparse. Form and markings as usual.

Antennae short, reaching the fifth segment composed of from 29 to 32, mostly very short, articles. Hairs moderate, straight, in part extending distoectad and in part almost directly transversely or somewhat caudoectad.

Eyes each composed mostly of about 8 or 9 to 11 ocelli in two or three series:—thus, 1+4, 4; 1+3, 3, 1; 1+4, 4, 2. Single ocellus considerably the largest. Seriate ocelli decreasing in size cephalad and ventrad.

Prosternum 1.58 times wider than long. Distance between chitinous spots 2 to 2.25 times greater than width at level of bottom of mesal incision; 3.75 to 4 times as great as the dental line. Teeth small; subacute; the interval between each pair concave; line of apices clearly recurved. Mesal incision rather large, sides but little converging caudad, the bottom being rather widely rounded. Spine slender and acuminate; inserted a little ectad of outer tooth and

extending somewhat mesad of directly cephalad. Lateral margin ectad of spine, convex, more caudad becoming concave and bending ectad.

Coxal pores small and circular; 2, 3, 3, 2 to 3, 4, 4, 3.

Spines of first legs, $\frac{0, 0, 1, 1, 1}{0, 0, 0, 1, 1}$ or $\frac{0, 0, 1, 1, 1}{0, 0, 0, 2, 1}$; of the second to fifth inclusive, $\frac{0, 0, 2, 2, 1}{0, 0, 0, 2, 1}$; of the sixth and seventh, $\frac{0, 0, 2, 2, 2}{0, 0, 0, 2, 1}$; of the eighth and ninth, $\frac{0, 0, 2, 2, 2}{0, 0, 1, 2, 1}$; of the tenth and eleventh, $\frac{0, 0, 3, 2, 2}{0, 0, 1, 2, 1}$; of the twelfth, $\frac{0, 0, 3, 1, 1}{0, 0, 2, 3, 1}$; of the thirteenth, $\frac{0, 0, 3, 1, 1}{0, 1, 3, 3, 1}$; of the penult, $\frac{0, 0, 3, 1, 0}{0, 1, 3, 3, 1}$ or $\frac{0, 0, 2, 1, 0}{0, 1, 3, 3, 1}$; claws 2 (or with obsolete third one); of the anal, $\frac{0, 0, 2, 0, 0}{0, 1, 3, 2, 0}$, claws 2 (in regenerated legs may be but 1).

None of the coxae at all armed.

Anal legs of male short and a little inflated; the third, fourth and fifth, but most distinctly the fourth, joints longitudinally sulcate on dorsal side. Penult legs similar but a little more slender.

Claw of the female gonopods short and relatively wide; tripartite; the median lobe but little longer than the two lateral, the distal margin of the latter almost at right angles to the longitudinal axis of claw. Basal spines very broad at base as usual, the sides parallel or a little diverging to about middle of length, then converging to an acute point. Inner side of first article nearly straight, being a little convex distally and slightly concave proximally, nearly parallel to that of its opposite gonopod.

Length 7-9 mm. A female 8 mm. long has the tenth plate .98 mm. wide; antenna 4 mm. long; and the anal leg 3.8-4 mm. long.

Praematurus.—Coloration similar to that of adult but paler throughout; light brown of a pale violaceous caste, with the fourteenth plate darker and the fifteenth lighter, the latter being yellowish as in the adult. Head light orange. Antennae brown, light at tips. Prosternum yellowish. Venter pale, the first plates more strongly tinged with violaceous, and the last one more strongly pigmented, yellowish or light orange. Legs pale, suffused with very dilute violaceous, the last pairs light yellow excepting proximally.

Antennae composed of twenty-five articles of which those beyond the third or fourth are very short and compactly arranged and those near the middle of length the shortest. Hairs straight, of moderate length, not dense.

Eyes composed of seven or eight ocelli arranged in two series:—thus, 1+4, 3; 1+4, 2. Ocelli small and pale, the single one largest and the most anterior ones smallest.

Prosternum is in adult or nearly so.

Coxal pores very small; 2, 3, 3, 3.

Spines of first legs, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 0, 1}$; of the second, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 0, 1}$ to $\frac{0, 0, 0, 2, 1}{0, 0, 0, 1, 1}$; of the third to sixth, $\frac{0, 0, 0, 2, 1}{0, 0, 0, 0, 1}$; of the seventh, $\frac{0, 0, 1, 2, 1}{0, 0, 0, 2, 1}$; of the eighth, $\frac{0, 0, 1, 2, 2}{0, 0, 0, 2, 1}$ or $\frac{0, 0, 2, 2, 2}{0, 0, 0, 2, 1}$; of the ninth, $\frac{0, 0, 2, 2, 2}{0, 0, 0, 2, 1}$; of the tenth and eleventh, $\frac{0, 0, 2, 2, 2}{0, 0, 0, 2, 1}$ or $\frac{0, 0, 2, 2, 2}{0, 0, 1, 2, 1}$; of the twelfth, $\frac{0, 0, 2, 1, 1}{0, 0, 1, 2, 1}$ or $\frac{0, 0, 2, 1, 1}{0, 0, 1, 3, 1}$; of the thirteenth, $\frac{0, 0, 2, 1, 1}{0, 1, 2, 3, 1}$; of the penult, $\frac{0, 0, 2, 1, 0}{0, 1, 3, 3, 1}$; of the anal, $\frac{0, 0, 2, 0, 0}{0, 1, 3, 2, 0}$.

Claw of female gonopods short and relatively wide, with the three divisions acute and the outermost somewhat smallest. Spines 2+2; the outer one of each pair of nearly adult form, being broad or somewhat clavate proximally and acuminate from near the middle of length, the apex very narrowly rounded, the inner spine about half the size of the outer one, acuminate from very base.

Length cir. 5.25 mm. Length of antennae 2 mm.; of anal legs, cir. 2.2 mm.

(Haddonfield, N. J., Oct. 27, 1912).

Immaturus.—Still paler than the *prematurus*; of a violaceous tinge throughout; the last dorsal slate yellow. Head and prosternum yellow. Anal and penult legs with tibiae and tarsi yellow, the more proximal joints having the pale violaceous tinge of the rest of the legs.

Antennae very short. Composed of nineteen articles which distad of the third are very short. Hairs about as in the *prematurus*.

Eyes composed of about six ocelli arranged in two series:—thus, 1+3, 2. The most caudal ocellus of the dorsal series largest, the single one next, with the anterior one and those of the lower series much smaller.

Prosternum essentially as in older stages.

Coxal pores very small and pale; 2, 2, 2, 2.

Spines of first to third pairs of legs, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 0, 1}$; of the fourth to sixth pairs, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 1, 1}$; of the seventh to ninth pairs, $\frac{0, 0, 0, 2, 1}{0, 0, 0, 1, 1}$; of the tenth and eleventh pairs, $\frac{0, 0, 0, 2, 2}{0, 0, 0, 1, 1}$; of the twelfth, $\frac{0, 0, 1, 1, 1}{0, 0, 1, 2, 1}$; of the thirteenth, $\frac{0, 0, 2, 1, 1}{0, 0, 1, 2, 1}$ or $\frac{0, 0, 2, 1, 1}{0, 1, 1, 2, 1}$; of the penult, $\frac{0, 0, 2, 1, 0}{0, 1, 2, 2, 1}$; of the anal, $\frac{0, 0, 2, 0, 0}{0, 1, 3, 1, 0}$.

Gonopods of female very short and pale; the three articles present, but the division between the second and third not yet very distinct. Claw indicated as a mere spinous point, the article beneath it distally

well rounded and bearing also a single bristle. Second article likewise bearing a single bristle. Proximal article bearing three bristles in a transverse row. Spines 1+1, small and pale; acutely acuminate from the very base.

Length cir. 4.2 mm.

(Haddonfield, N. J., Oct. 27, 1912).

TYPE LOCALITY.— North Carolina (Hot Springs).

LOCALITIES.— North Carolina (Hot Springs!); Alabama (Birmingham!); New Jersey (Haddonfield!).

TIDABIUS POAPHILUS, sp. nov.

DIAGNOSIS.— Ventral spines of anal legs 0, 1, 3, 2, 0. Dorsal spines of penult legs, 0, 0, 3, 1, 0. Third joint of tenth to fourteenth legs bearing 3 dorsal spines. Ventral spines of tenth and eleventh legs, 0, 0, 2, 3, 1. Ventral spines of the first legs, 0, 0, 1, 1, 1, or, occasionally, 0, 0, 0, 1, 1. None of the coxae armed.

Articles of antennae 32.

Ocelli 9–12 in three series.

Distance between chitinous spots about 2.25 times the width at median incision; 4 times the dental line.

Coxal pores 3, 4, 4, 3.

Head wider than long.

Body moderately robust, 7.5–7.75 times longer than the width of the tenth plate.

Claw of female gonopods as typical in the genus. Spines broad proximally, the sides of this portion parallel or a little diverging; acuminate portion more than half the total length.

Dorsum brown or greyish brown, suffused with dark purplish; the first and last several plates darker. Head brown to orange-brown, with the frontal region lightest.

DESCRIPTION.— Above brown or greyish brown, commonly suffused with dark purple; the first and the last several plates darkest. Head brown or orange-brown, the frontal region lightest. Antennae light brown, lighter at tips, often tinged with purplish. Prosternum and prehensors brown. Venter greyish or dilute greyish brown, the anterior plates typically tinged with purple and the caudal plates darker, brown. Legs like the corresponding plates of venter, the first commonly with a purplish tinge and the caudal pairs brownish proximally, brighter, yellowish, distally.

Body moderate or rather robust; the type 7.65 times longer than the width of the tenth plate. Widths of head and of first, third, eighth, tenth, and twelfth plates to each other as 23: 21: 23: 26: 27: 26.

Head wider than long in ratio 50: 47.5. Caudal margin slightly and widely excurved. Sides between eyes and marginal interruptions very little excurved, subparallel. Hairs long, sparse.

Antennae of the usual length. Composed of 32 articles which beyond the second are very short and closely united, submoniliform. Hairs of moderate length and short; not very dense, sparser proximad; extending in various directions as usual.

Eyes each composed of 9-11 ocelli arranged in three, or occasionally, in but two, series:—*e. g.*, 1+4, 3, 3; 1+1, 4, 3; 1+5, 3. The lowest series almost on ventral side of head. Single ocellus largest. Of the seriate ocelli the first or most caudal of the uppermost series is largest, the first of the second series also large; others decreasing cephalad and ventrad as usual.

Prosternum 1.64 times wider than long. Distance between chitinous spots 2.25 times width at level of bottom of median incision; 4 times as great as the length of the dental line. Median incision moderately large, sides gently converging caudad; rather widely rounded at bottom. Teeth small, acute, equal; line of apices recurved. Tubercle of spine contiguous with base of outer tooth as usual; spine rather stout at base, running to a fine point with sides straight throughout length or nearly so, extending directly cephalad or nearly so, as in *T. anderis*. Lateral margins of anterior portions sloping directly caudoectad immediately from base of spine; the margins strongly incurved with caudal portion almost transverse. (Plate 5, fig. 3).

First dorsal plate with sides diverging cephalad to about the middle and then parallel or nearly so. 1.8+ times wider than long. Caudal angles of thirteenth plate a little produced as usual; angles of eleventh rectangular or showing a slight tendency to production; other minor plates with caudal margins bending a little forward at ends about the rounded corners. All plates finely but distinctly roughened with tubercular and rugose elevations.

Coxal pores small, circular; 3, 4, 4, 3; on each coxa decreasing in size proximad with the most proximal often very small.

Spines of first legs, $\frac{0, 0, 1, 1, 1}{0, 0, 1, 1, 1}$ or $\frac{0, 0, 1, 1, 1}{0, 0, 0, 1, 1}$, of the second, $\frac{0, 0, 2, 2, 1}{0, 0, 1, 2, 1}$ or $\frac{0, 0, 2, 2, 1}{0, 0, 0, 2, 1}$; of the third to fifth inclusive, $\frac{0, 0, 2, 2, 1}{0, 0, 1, 2, 1}$; of the sixth, $\frac{0, 0, 2, 2, 2}{0, 0, 1, 2, 1}$; of the seventh and eighth, $\frac{0, 0, 2, 2, 2}{0, 0, 1, 2, 1}$, or on

one side this and on the other, $\frac{0, 0, 2, 2, 2}{0, 0, 0, 2, 1}$; of the ninth, $\frac{0, 0, 2, 2, 2}{0, 0, 1, 3, 1}$, or $\frac{0, 0, 2, 2, 2}{0, 0, 1, 3, 1}$; of the tenth and eleventh, $\frac{0, 0, 3, 2, 2}{0, 0, 2, 3, 1}$; of the twelfth, $\frac{0, 0, 3, 1, 1}{0, 0, 2, 3, 1}$; of the thirteenth, $\frac{0, 0, 3, 1, 1}{0, 1, 3, 3, 1}$; of the penult, $\frac{0, 0, 3, 1, 0}{0, 1, 3, 3, 1}$; claws 3; of the anal, $\frac{0, 0, 2, 0, 0}{0, 1, 3, 2, 0}$, claws 2. None of the coxae at all armed.

Posterior legs but little, uniformly inflated. Third, fourth, and fifth articles longitudinally furrowed on dorsal side as usual. Not differing in male and female.

Gonopods of the male small and rounded; bearing 1 or 2 bristles.

Claw of female gonopods short and but slightly curved; distinctly tripartite in the usual way; median lobe longest, obtusely rounded, less acuminate than in *anderis*; lateral lobes with distal margins nearly at right angles to axis of claw. Basal spines broad at base as in related species; the sides proximally parallel or a little diverging distad; acuminate portion long, more than half the total length. Mesal margin of first joint nearly straight, a little diverging from its fellow cephalad. Sternite mesally a little incurved as in related species and without a distinctly chitinized process.

The ♂ type is 7.5 mm. long; width of tenth dorsal plate .98 mm.; length of antenna 2.5 mm.; of anal leg, 3 mm.

TYPE LOCALITY.—Nebraska (Fremont).

LOCALITIES.—Nebraska (Fremont! Omaha!).

TIDABIUS ANDERIS, sp. nov.

DIAGNOSIS.—Ventral spines of anal legs 0, 1, 3, 2, 0. Dorsal spines of penult legs, 0, 0, 3, 1, 1. Third joint of twelfth to fourteenth pairs of legs inclusive with three dorsal spines. Ventral spines of tenth and eleventh legs, 0, 0, 1, 2, 1. Ventral spines of first legs 0, 0, 0, 1, 1. None of the coxae armed.

Articles of antennae (in type) 31.

Ocelli 8 in two series.

Distance between chitinous spots 4 times the dental line; 2.46 times width at median incision.

Coxal pores 3, 4, 4, 3.

Claw of female gonopods as typical in the genus. Spines broad at base as in *T. tivius* but the acuminate apical portion embracing considerably more than half the total length.

Head longer than wide.

Body robust, being only about 7.4 times longer than width of tenth plate.

Dorsum testaceous, usually of dilute olivaceous or, sometimes, of somewhat purplish tinge; first and last plates darker as usual. Head yellowish orange to light ferruginous.

DESCRIPTION.—Above testaceous, in some of dilute olivaceous tinge, in others more or less purplish; the first plate and the most caudal ones often darker and some of the plates with their caudal borders crossed by a transverse dark stripe. Head yellowish orange to light ferruginous with a dark area on caudal portion along margin and extending forward along median line part way to the suture. Antennae brownish proximally, becoming yellow distad. Prosternum and prehensorial feet yellow to brown, the prosternum usually dusky over a yellow background. Venter pale, dilute testaceous or yellowish, the caudal plates darker brown. Legs like the contiguous plates of venter excepting that the posterior pairs beyond the proximal portion are bright yellow.

Body robust; in ♀ type, 7.4 times longer than width of tenth plate. Widths of head and of the first, third, eighth, tenth, and twelfth plates to each other as 25:23:24:28:26.5.

Head longer than wide in ratio 50:47; a little more elongate than in *T. tivius* with the anterior portion somewhat narrower; not much varying in width between the marginal breaks and eyes; caudal margin straight.

Antennae short or very short. Composed of 31 articles which are very short, especially distad, and are closely aggregated. Bristles moderate in length, subdense on distal articles, becoming considerably sparser proximad.

Eyes in type each composed of about 8 ocelli arranged in two series:—thus, 1+4, 3. Ocelli large and distinct; single ocellus somewhat obliquely elliptic.

Prosternum 1.64 times wider than long. Distance between chitinous spots 2.46 times width at level of bottom of median incision; 4 times as great as length of the dental line. Teeth small and acute, pale; line of apices recurved. Tubercle of spine contiguous with base of outer tooth; spine manifestly stouter than the hairs, acutely acuminate as usual, extending almost directly cephalad and thus not parallel with outer edge of tooth as is more typically the case in *tivius*. Median incision rather narrow, the sides nearly parallel, but little converging caudad excepting toward bottom, the latter somewhat rounded.

First plate moderately narrowed caudad as usual, 1.9 times wider than long. Minor plates as in *tivius* or nearly so; in the anterior ones the caudal margin moderately bending forward to corners at ends; in the eleventh the posterior corners are nearly rectangular; in the thirteenth they are a little extended caudad. Plates all distinctly finely roughened.

Coxal pores small, circular, the most proximal one on some coxae minute; 3, 4, 4, 3.

Spines of first legs, $\frac{0, 0, 1, 1, 1}{0, 0, 0, 1, 1}$; of the second to sixth inclusive, $\frac{0, 0, 2, 2, 1}{0, 0, 0, 2, 1}$; of the seventh to ninth inclusive, $\frac{0, 0, 2, 2, 2}{0, 0, 0, 2, 1}$; of the tenth and eleventh, $\frac{0, 0, 2, 2, 2}{0, 0, 1, 2, 1}$; of the twelfth and thirteenth, $\frac{0, 0, 3, 1, 1}{0, 0, 2, 3, 1}$; of the penult, $\frac{0, 0, 3, 1, 1}{0, 1, 3, 3, 1}$; of the anal, $\frac{0, 0, 2, 0, 0}{0, 1, 3, 2, 0}$, claws 2. No spines detected on any coxae.

Anal and penult legs in both sexes short; a little thickened.

Claw of female gonopods short; but little curved; median lobe longest, distally more or less narrowly rounded; lateral teeth with their distal edges in line and at right angles to the axis of the claw or nearly so. Spines broad at base as in *tivius*, but the acuminate portion longer, embracing considerably more than half the total length, apically acutely rounded with edge often showing a few minute denticulations. Ventral edge of mesal side of first joint well chitinized; nearly straight excepting proximally where it bends a little ectad.

The female type is 7.4 mm. long; width of tenth plate 1 mm.; length of antenna 2.25 mm.

LOCALITY.—Washington (Pullman! W. M. Mann, 1908).

TIDABIUS PALLIDUS, sp. nov.

DIAGNOSIS.—Ventral spines of anal legs 0, 1, 3, 1, 0. Dorsal spines of penult legs 0, 0, 3, 1, 0. Third joint of tenth to fourteenth legs with three dorsal spines. Ventral spines of tenth and eleventh legs, 0, 0, 2, 3, 1. Ventral spines of first legs, 0, 0, 0, 1, 1. None of the coxae armed.

Articles of antennae, in type, 27.

Ocelli 9 or 10 in three series.

Distance between chitinous spots of prosternum 3.5 times the dental line; only 2.1 times width at median incision.

Head cordate, very little wider than long.

Coxal pores 3, 4, 4, 3.

Body moderately slender, about nine times longer than the width of the tenth plate.

Spines of ♀ gonopods large and broad, the sides parallel to near middle from where the acutely acuminate portion begins.

Dorsum yellow. Head light orange.

DESCRIPTION.— Yellow above with the caudal plates light orange. Head light orange. Antennae pale. Prosternum yellow of pale orange tinge. Venter very pale with caudal plates orange. Legs pale, the posterior pairs more densely pigmented, brighter in color.

Body slender, being 9 times longer than the width of the tenth plate. Widths of head and of first, third, eighth, tenth, and twelfth plates to each other as 45: 40: 43: 51: 52: 48.

Head cordate; slightly wider than long (ratio about 45.5: 44). Hair short, sparse.

Antennae short and moderately attenuated as usual. In type composed of 27 articles which beyond the second are very short, shorter ones occurring in pairs at intervals among somewhat longer ones. Hairs as usual.

Eyes in type each composed of 9 or 10 ocelli arranged in three series:— thus, 1+4, 3, 1; 1+4, 3, 2. Single ocellus largest; first ocellus of top row also large and the others of this series also larger than the lower ones which decrease in size ventrad.

Prosternum 1.58 times wider than long. Distance between chitinous spots 2.1 times the width at level of bottom of median incision; 3.5 times the dental line. Median incision wide; sides distally concave, meeting at bottom in a wide angle; distance from tip to tip of mesal teeth clearly greater than between tips of teeth of each pair. Teeth small and acute; line of apices slightly recurved. Anterior lateral margin at first strictly transverse ectad of each outer tooth for a short distance, this portion bearing the spine, the caudally sloping portion at first conspicuously convex, becoming caudally concave and at caudal end bending directly ectad. Spine conspicuously stout at base, slender and fine pointed distally.

First dorsal plate 1.78 times wider than long, shaped as in *T. nasinius*. Caudal corners of thirteenth plate a little produced as usual; those of the eleventh caudally straight or somewhat excised; those of the ninth mostly a little excised or with caudal edge bent forward.

Coxal pores of the usual size and form; 3, 4, 4, 3.

Spines of first legs, $\frac{0}{0}, \frac{0}{0}, \frac{1}{1}, \frac{1}{1}$; of the second to seventh inclusive, $\frac{0}{0}, \frac{0}{0}, \frac{2}{2}, \frac{2}{2}, \frac{2}{2}, \frac{2}{2}$; of the ninth, $\frac{0}{0}, \frac{0}{0}, \frac{1}{1}, \frac{1}{1}$; of the tenth and eleventh, $\frac{0}{0}, \frac{0}{0}, \frac{2}{2}, \frac{1}{1}$.

$\frac{0, 0, 3, 2, 2}{0, 0, 2, 3, 1}$; of the twelfth, $\frac{0, 0, 3, 1, 1}{0, 0, 2, 3, 1}$; of the thirteenth, $\frac{0, 0, 3, 1, 1}{0, 1, 3, 3, 1}$; of the penult, $\frac{0, 0, 3, 1, 0}{0, 1, 3, 3, 1}$, claws 2; of the anal, $\frac{0, 0, 2, 0, 0}{0, 1, 3, 1, 0}$, claws 2.

None of the coxae armed.

Posterior legs as usual.

Claw of female gonopods relatively short and wide as usual; lobes short but distinct, the median longest, the two lateral subequal and at same level with their caudal edges almost transverse. Spines large, broad, with sides from base to about middle parallel, spines distad from middle acutely acuminate. Inner side of first article diverging moderately from its fellow proximally.

The female type is 8.7 mm. long; width of tenth plate .97 mm.; length of antenna 3 mm.; of anal leg, 2.7 mm.

LOCALITY.—Mississippi (Jackson!).

TIDABIUS PALLIDUS ALABAMENSIS, subsp. nov.

DIAGNOSIS.—Light brown, with the first and last plates darker. Head brown of dilute ferruginous tinge.

Somewhat more robust than type of *T. pallidus*, being but 8.5 times longer than width of the tenth plate.

Spining of legs as in *pallidus* or essentially so.

Coxal pores 3, 4, 4, 4.

Head as in *pallidus*.

Gonopods and spines nearly as in *pallidus*.

Distance between chitinous spots 3.1 times the dental line.

DESCRIPTION.—Dorsum light brown, the first two or three plates and, more especially, those of the caudal third distinctly darker. Head brown of pale ferruginous tinge. Antennae brown proximally, pale distally. Prosternum and prehensorial feet brown. Venter pale, the anterior plates suffused with violaceous, the posterior plates darker, brownish. Legs like adjoining plates of venter; the posterior pairs brown proximally and bright yellow or yellow of dilute orange tinge distally.

Body 8.5 times longer than width of the tenth plate. Widths of head and of the first, third, eighth, tenth, and twelfth plates to each other as 42:40:42:47:50:48.

Head a little wider than long (near 42:41); widest immediately back of eyes, with the sides conspicuously converging from the breaks caudad.

Antennae in type broken off at tips so precise number of articles is not determinable.

Eyes each composed of 12 ocelli in three series:—thus, 1+4, 4, 3; ocelli of the usual relative sizes and arrangement.

Prosternum 1.55+ times wider than long. Distance between chitinous spots about 2 times width at level of bottom of median incision; 3.1 times the dental line. Median incision wide, well rounded at bottom; distance from tip to tip of mesal teeth clearly greater than distance between tips of tenth of each pair. Teeth small and acute; line of apices almost straight, being only very slightly recurved. Sides of anterior portion convex anteriorly and bending ectad caudally as usual. Spine of usual form.

First dorsal plate of the usual shape, slightly shorter relatively than in type of *pallidus*. Thirteenth plate with posterior angles weakly produced; other minor plates with caudal corners more or less obliquely excised.

Coxal pores of usual size and shape; 3, 4, 4, 4.

Spines of first legs, $\frac{0, 0, 2, 1, 1}{0, 0, 0, 1, 1}$; of the second to fifth, $\frac{0, 0, 2, 2, 1}{0, 0, 0, 2, 1}$; of the sixth to eighth, $\frac{0, 0, 2, 2, 2}{0, 0, 0, 2, 1}$; of the ninth, $\frac{0, 0, 2, 2, 2}{0, 0, 1, 2, 1}$; of the tenth, $\frac{0, 0, 3, 2, 2}{0, 0, 2, 2, 1}$ or $\frac{0, 0, 3, 2, 2}{0, 0, 1, 2, 1}$; of the eleventh, $\frac{0, 0, 3, 2, 2}{0, 0, 2, 3, 1}$; of the twelfth, $\frac{0, 0, 3, 1, 1}{0, 0, 2, 3, 1}$; of the thirteenth, $\frac{0, 0, 3, 1, 1}{0, 1, 3, 3, 1}$; of the penult, $\frac{0, 0, 3, 1, 0}{0, 1, 3, 3, 1}$; claws 2; of the anal, $\frac{0, 0, 2, 0, 0}{0, 1, 3, 1, 0}$; claws 2, long. None of the coxae armed.

Claw of female gonopods short and relatively wide; lobes short, with the median only a little longest, the two lateral equal and at the same level. Spines broad proximally, the sides diverging from base to a little below middle, the spine then acutely long acuminate. Inner side of first article subparallel with that of its mate excepting proximally, where it moderately diverges from it.

Length of female type 8 mm.; width of tenth plate .94—mm.

LOCALITY.—Alabama (Selma!).

While manifestly very close to *pallidus*, the type of *alabamensis* is strikingly different in color, being much more in appearance like *nasintus*. Otherwise it differs a little also in robustness, being a little stouter. The prosterna are very similar. As only the type of each form has been examined, the precise relationship of the two cannot at present be definitely determined; but it seems best to keep them apart tentatively and to range one as subspecies to the other.

TIDABIUS TIVIUS (Chamberlin).

Lithobius tivius Chamberlin, Ann. Ent. soc. America, 1909, 2, p. 188.

DIAGNOSIS.—Ventral spines of anal legs 0, 1, 3, 1, 0. Dorsal spines of penult legs, 0, 0, 3, 1, 0. Third joint of tenth to fourteenth legs armed dorsally with three spines. Ventral spines of eleventh legs, 0, 0, 2, 2, 1; ventral spines of first legs, 0, 0, 0, 0, 1. None of the coxae armed.

Articles of antennae 25 to 31.

Ocelli mostly 9 to 12 in three series.

Distance between chitinous spots 3.8 to 4.25 times the dental line; 2.3 times width at median incision.

Head equal in length and breadth or very nearly so.

Body robust, from 7.25 to 8.25, or thereabouts, times longer than the width of the tenth plate.

Coxal pores 2, 3, 4, 3-3, 4, 4, 3.

Claw of gonopods as usual. Spines broad with sides diverging to about the middle where the acuminate distal portion begins. (Plate 4, fig. 5).

Dorsum pale brown or of pearl-grey caste, with the caudal plates more or less of a ferruginous tinge. Head light ferruginous.

DESCRIPTION.—Above pale brown faintly suffused with violaceous or sometimes of pearl-grey caste; the caudal plates somewhat ferruginous. Head light ferruginous, commonly darkened over median region of caudal portion. Antennae brown to somewhat ferruginous proximally, lighter distad. Prosternum paler than head, the prehensorial feet lighter. Venter very pale, suffused with violaceous; the caudal plates darker, more or less of ferruginous tinge. Legs also pale, the caudal pairs darker, brown, proximally, and brighter, yellowish, distad.

Body robust, being about 7.25 to 8.25 times longer than the width of the tenth plate. Widths of head and of first, third, eighth, tenth, and twelfth plates to each other as 40:34:37:40:43:42.

Head subcordate; equal in length and breadth or very nearly so. Hairs moderately long with shorter ones intermixed, sparse.

Antennae short and moderately attenuated. Composed of 25 to 31 articles which distad of the second are very short. Bristles almost transverse not especially dense. (Plate 5, fig. 6).

Eyes each consisting of 10 or 11 ocelli in the types:—*e. g.*, 1+4, 4, 2; 1+4, 3, 2. Single ocellus largest; others regular in form and arrangement. (Plate 5, fig. 7).

Prosternum 1.58 times wider than long. Distance between chitinous spots 2.3 times width at level of bottom of median incision; 3.8 to 4.25 times the dental line. Median incision v-shaped, with sides rather strongly converging. Teeth small and acute; interval between the two on each side semicircular; line of apices clearly recurved. Anterior lateral margins rather strongly convex anteriorly, concave caudally. (Plate 5, fig. 2-3).

First dorsal plate 1.8 times wider than long; widest a little behind the anterior end where the sides are convex; sides then nearly straight and moderately converging to the rounded caudal corners. Posterior angles of the eleventh and thirteenth plates a little extended caudad, rounded, those of ninth straight or obliquely excised.

Coxal pores small and circular as usual; 2, 3, 4, 3 - 3, 4, 4, 3.

Spines of first legs, $\frac{0, 0, 1, 1, 1}{0, 0, 0, 0, 1}$ or $\frac{0, 0, 1, 1, 1}{0, 0, 0, 1, 1}$; of the second, $\frac{0, 0, 2, 2, 1}{0, 0, 0, 1, 1}$ or $\frac{0, 0, 2, 2, 1}{0, 0, 0, 2, 1}$; of the third to fifth, $\frac{0, 0, 2, 2, 1}{0, 0, 0, 2, 1}$; of the sixth, $\frac{0, 0, 2, 2, 1}{0, 0, 0, 2, 1}$ or $\frac{0, 0, 2, 2, 2}{0, 0, 0, 2, 1}$; of the seventh to ninth, $\frac{0, 0, 2, 2, 2}{0, 0, 0, 2, 1}$; of the tenth, $\frac{0, 0, 3, 2, 2}{0, 0, 1, 2, 1}$; of the eleventh, $\frac{0, 0, 3, 2, 2}{0, 0, 2, 2, 1}$ or $\frac{0, 0, 3, 2, 2}{0, 0, 1, 2, 1}$; of the twelfth, $\frac{0, 0, 3, 1, 1}{0, 0, 2, 3, 1}$; of the thirteenth, $\frac{0, 0, 3, 1, 1}{0, 1, 2, 3, 1}$ to $\frac{0, 0, 3, 1, 1}{0, 1, 3, 3, 1}$; of the penult, $\frac{0, 0, 3, 1, 0}{0, 1, 3, 3, 1}$; of the anal, $\frac{0, 0, 2, 0, 0}{0, 1, 3, 1, 0}$, claws 2. None of the coxae armed.

Anal and penult legs (♀) short; uniformly a little inflated. (Plate 5, fig. 4-5).

Claw of gonopods of female moderate; relatively wide; but weakly curved; the median lobe longest and the lateral ones equal and at the same level as usual. Basal spines broad proximally; sides diverging a little from base to about middle where the acutely acuminate distal portion begins, the sides of the latter straight. Mesal side of first article moderately diverging in proximal half from its fellow. (Plate 4, fig. 5).

Length 5.5-7 mm. A specimen 5.8 mm. in length has tenth plate .8+ mm. wide; and antenna 2 mm. long.

Pseudomaturus.—Freshly matured individuals seem to agree in nearly all features with the older ones; but the sixth legs more frequently bear but a single dorsal tibial spine instead of two and the eleventh legs more frequently bear a single ventral spine on the pre-femur instead of two. Thus the spining, as might be expected, more frequently remains in the condition prevalent in the *praematurus* stage.

Praematurus.—Dilute brown, suffused more or less with violaceous, this being evident more especially on the anterior and posterior seg-

ments both above and below. Head light ferruginous. Posterior legs as usual more pigmented than the others.

Antennae composed of twenty-six or twenty-seven articles of nearly adult proportions.

Ocelli six, in two series:—thus, 1+3, 2. The single ocellus and the first of the upper row of the same size or nearly so and much larger than the others, the most anterior smallest.

Prosternum as in the adult.

Coxal pores small and pale; 2, 3, 3, 2.

Spines of the first legs, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 0, 1}$; of the second pair, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 1, 1}$ or $\frac{0, 0, 0, 2, 1}{0, 0, 0, 1, 1}$; of the third to sixth, $\frac{0, 0, 0, 2, 1}{0, 0, 0, 1, 1}$; of the seventh, $\frac{0, 0, 1, 2, 1}{0, 0, 0, 2, 1}$; of the eighth and ninth, $\frac{0, 0, 2, 2, 1}{0, 0, 0, 1, 1}$; of the tenth and eleventh, $\frac{0, 0, 2, 2, 2}{0, 0, 1, 2, 1}$; of the twelfth, $\frac{0, 0, 3, 1, 1}{0, 0, 1, 2, 1}$; of the thirteenth, $\frac{0, 0, 2, 1, 1}{0, 0, 2, 3, 1}$; of the penult, $\frac{0, 0, 2, 1, 1}{0, 1, 3, 3, 1}$; of the anal, $\frac{0, 0, 2, 0, 0}{0, 1, 3, 1, 0}$.

Claw of the female gonopods small and pale, the three lobes distinct but appearing more slender and acute than in the adult. Proximal article with about six bristles on its ventral surface. Spines 2+2; the inner one of each pair shorter and much more slender than the outer and acutely acuminate from the very base; in the outer spine also the demarcation between basal and acuminate portions less distinct than in the adult.

(Canton, Miss.).

Agentialis I.—Very dilute yellowish, with the head as usual more densely pigmented. Antennae and legs pale; the last pairs of legs with more pigment, very pale yellowish.

Antennae composed of from twenty to twenty-three articles. Submoniliform. The last article conspicuously long. Hairs straight; of moderate length; not dense.

Ocelli four in one or two series:—thus, 1+3, or 1+2, 1. Small and pale.

Teeth of prosternum pale, their form and relations, as those of the ectal spines, as in the adult or nearly so.

Coxal pores small and weak edged; 1, 1, 1, 1 or 1, 2, 2, 1, the more proximal one when two are present on a coxa being considerably smallest.

Anal glands still evident but not very distinct.

Spines of first and second pairs of legs, $\frac{0, 0, 0, 0, 1}{0, 0, 0, 0, 1}$; of the third to ninth, $\frac{0, 0, 0, 1, 1}{0, 0, 0, 1, 1}$; of the tenth to thirteenth, $\frac{0, 0, 0, 0, 0}{0, 0, 0, 1, 1}$; of the penult, $\frac{0, 0, 0, 0, 0}{0, 0, 1, 1, 1}$; of the anal, $\frac{0, 0, 0, 0, 0}{0, 0, 1, 1, 0}$.

(Sterling, Ill., July).

TYPE LOCALITY.—Utah (Provo!).

LOCALITIES.—Utah (Provo! Salt Lake City!); Colorado (Colorado Springs! Ft. Collins!); Illinois (Sterling! Dwight! Peoria!); Kentucky (Lexington!); Tennessee (Johnson City!); Virginia (variety, Lynchburg! Balcony Falls!); New Jersey (Haddonfield!); Pennsylvania (Philadelphia! Ardmore!); Wisconsin (Janesville!); Massachusetts (Hough's Neck!); Alabama (Birmingham!); Mississippi (Brookhaven! Canton!).

TIDABIUS OPIPHILUS, sp. nov.

DIAGNOSIS.—Ventral spines of anal legs 0, 1, 3, 1, 0. Dorsal spines of penult legs 0, 0, 3, 1, 0. Third joint of tenth to fourteenth pairs of legs with three dorsal spines. Ventral spines of eleventh legs, 0, 0, 1, 3, 1. Ventral spines of first legs, 0, 0, 0, 1, 1.

Articles of antennae 27 to 32.

Ocelli 9 to 12 in three or, rarely, in four longitudinal series.

Head subcordate; wider than long.

Distance between chitinous spots 3.7 times as great as the dental line; 2.3 times the width at median incision.

Body 9 to 9.5 times longer than the width of the tenth plate.

Claw of the female gonopods much as usual. Basal spines broad proximally with sides subparallel; acuminate portion about two thirds the total length. (Plate 5, fig. 9).

Brown; first and last plates darker. Head dark brown of a dilute ferruginous tinge.

DESCRIPTION.—Dorsum brown; first and caudal plates darker; the median region pale. Head darker, brown of a dilute ferruginous tinge, lightest in front of suture. Antennae brown proximally, lighter, yellowish or pale ferruginous, distally. Prosternum brown, the prehensorial feet paler. Venter very pale, tinged with violaceous anteriorly and the caudal plates darker, burnt brown. Legs pale like the venter; the caudal pairs darker, brown, commonly brighter distally.

Slender, moderately attenuated cephalad; 9 to 9.5 times longer than width of the tenth plate. Widths of head and of first, third, eighth, tenth, and twelfth plates to each other as 48:44:45:51:52:49.

Head subcordate, being considerably narrowed cephalad; wider than long in about ratio 49:47. Hairs of moderate length, sparse.

Antennae short. Composed of from 27 to 32 articles which are short and very short. Hairs rather short, subdense.

Eyes each composed of from 9 to 12 ocelli in three or four series:—*e. g.*, 1+4, 3, 2; 1+4, 3, 3; 1+4, 3, 3, 1.

Prosternum 1.56 times wider than long. Distance between chitinous spots 2.31 times width at level of bottom of median incision; 3.7 times the dental line. Median incision v-shaped; sides subparallel distad, concave and converging caudad. Teeth small and acute, the interval between the two of each pair well rounded at bottom, not at all angular. Spine a little ectad of tooth, straight and slender; extending directly cephalad. Anterior lateral margins convex distad then becoming concave and bending out almost directly ectad caudally.

First dorsal plate widest a little back of anterior end where the sides are rounded, becoming there nearly straight and gently converging to the rounded caudal corners; 1.9 times wider than long. Posterior angles of eleventh and thirteenth plates very weakly produced caudad; those of the ninth straight to more or less obliquely excised.

Coxal pores 3, 4, 4, 3-3, 3, 3, 3; often decreasing markedly in size proximal on each coxa, the most proximal then being minute.

Spines of first legs, $\frac{0, 0, 1, 1, 1}{0, 0, 0, 1, 1}$; of the second to fifth, $\frac{0, 0, 2, 2, 1}{0, 0, 0, 2, 1}$; of the sixth, $\frac{0, 0, 2, 2, 2}{0, 0, 0, 2, 1}$; of the seventh, $\frac{0, 0, 2, 2, 2}{0, 0, 0, 2, 1}$ or $\frac{0, 0, 3, 2, 2}{0, 0, 0, 2, 1}$; of the eighth, $\frac{0, 0, 3, 2, 2}{0, 0, 0, 2, 1}$; of the ninth, $\frac{0, 0, 3, 2, 2}{0, 0, 0, 2, 1}$ or $\frac{0, 0, 3, 2, 2}{0, 0, 1, 2, 1}$ (noted on one side only, the other leg having the first formula); of the tenth, $\frac{0, 0, 2, 2, 2}{0, 0, 1, 2, 1}$; of the eleventh, $\frac{0, 0, 3, 2, 2}{0, 0, 1, 3, 1}$; of the twelfth, $\frac{0, 0, 3, 1, 1}{0, 0, 2, 3, 1}$; of the thirteenth, $\frac{0, 0, 3, 1, 1}{0, 0, 2, 3, 1}$ or $\frac{0, 0, 3, 1, 1}{0, 1, 2, 3, 1}$; of the penult, $\frac{0, 0, 3, 1, 0}{0, 1, 3, 3, 1}$, claws 2; of the anal, $\frac{0, 0, 2, 0, 0}{0, 1, 3, 1, 0}$, claws 2. None of the coxae armed.

Anal legs short; uniformly but only a little thickened; longitudinally sulcate above, especially the fifth article. Penult legs similar, but a little more slender.

Claw of female gonopods very short; bent; median lobe longest and the lateral subequal as usual. Sides rather broad with sides of proximal division subparallel; acuminate portion rather long, being mostly about two thirds of the total length, the apex acute or narrowly acutely rounded. (Plate 5, fig. 9). Mesal side of first article more or less convex, diverging from its fellow proximately.

Length from 8 to 10 mm. A female 9 mm. long has tenth plate 1 mm. wide; antenna 2.8-3 mm. long; anal leg 3 mm. long.

LOCALITY.—Wisconsin (Beloit!).

TIDABIUS BONVILLENSIS (Chamberlin).

Lithobius bonvillensis Chamberlin, Ann. Ent. soc. America, 1909, 2, p. 189.

DESCRIPTION.—The original description of the types is as follows:—

Dorsum from light to blackish brown, the posterior border of most of the major plates usually lighter; head paler, brown or light brown, sometimes with a darker median area caudad of the frontal suture; antennae brown to blackish, yellowish or rufous distally; praesternum and feet concolorous with head; venter light brown to yellowish, the first and the last plates darkest; legs yellowish, the last few pairs commonly darker.

Head a little wider than long (3:2.9).

Antennae short; the first two articles long, others short, closely crowded; articles 29 in number.

Eyes each composed of 8–9 ocelli arranged in two series (1+4, 3; 1+5, 3); ocelli of moderate size.

Prosternal teeth small, slightly darkened.

Angles of none of the dorsal plates produced.

Coxal pores small, round, the distal ones of each series largest as usual; 3, 4, 4, 4.

Ventral spines of anal legs 0, 1, 3, 2, 0, claws 3. Ventral spines of penult legs, 0, 1, 3, 3, 1, claws 3. Ventral spines of first legs 0, 0, 0, 0, 1. None of the coxae at all armed.

Gonopods of female with the claw tripartite as usual, the lobes short and rather blunt. Basal spines 3+3; decreasing in size from the most ectal to the most mesal on each side; each spine acuminate distad of middle as usual.

LOCALITY.—Utah (Lake Point!).

This species was found in May under stones along the foot-hills.

SIGIBIUS, gen. nov.

Dorsal spines of anal legs 0, 0, 1, 0, 0; ventral, 0, 1, 1, 1, 0; claws 2. Dorsal spines of penult legs, 0, 0, 1, 0, 0; ventral, 0, 1, 1, 1, 0, claws 2. Dorsal spines of thirteenth legs, 0, 0, 1, 0, 0; ventral, 0, 0, 1, 1, 1. Dorsal spines of twelfth legs, 0, 0, 0, 0, 0; ventral, 0, 0, 0, 1, 1. Dorsal spines of eleventh legs, 0, 0, 0, 0, 1; ventral, 0, 0, 0, 1, 1. Dorsal spines of first legs, 0, 0, 0, 0, 1; ventral, 0, 0, 0, 0, 1. None of the coxae at all armed.

Antennae short; articles (in type) 25.

Eyes composed of few ocelli (6 or less in type) which are arranged in either one or two series; single ocellus decidedly largest as in *Tidabius*.

Prosternal teeth 2+2; line of apices rather strongly recurved.

Coxal pores circular; uniseriate.

Posterior legs of male short; uniformly a little thickened; without any special lobes or processes.

Claw of female gonopods in type nearly bipartite, the ectal lobe commonly obsolescent, then contracting with all known species of *Tidabius*. Basal spines moderately slender, acutely and evenly acuminate from very base to apex.

Posterior angles of none of the dorsal plates at all produced.

Small in size (max. length of type species cir. 7.5 mm.).

TYPE.—*S. puritanus*, sp. nov.

DISTRIBUTION.—New England States.

Evidently this genus is closest to the preceding one, *Tidabius*. From this genus, however, it is clearly distinct in the spining of the legs; *e. g.* the dorsal spines of the anal legs being 0, 0, 1, 0, 0 whereas in all species of *Tidabius* the corresponding formula is 0, 0, 2, 0, 0; also the ventral spines of these legs are 0, 1, 1, 1, 0, whereas in the other genus the formula is 0, 1, 3, 1, 0 or, more rarely, 0, 1, 3, 2, 0; and the contrast in spining of the penult legs is still greater and equally constant. The prosternum is similar to that of *Tidabius* though the line of apices of teeth is more strongly recurved than in any species of the latter genus. The basal spines of the female gonopods are conspicuously different in shape, being more slender and not showing any division into broader proximal portion, the sides of which are divergent or parallel, and an acuminate distal portion, so characteristic in all species of *Tidabius*, but attenuated uniformly from base to apex; etc. The ocelli are fewer in number and show a pronounced tendency to form but a single series. *Tidabius* and *Sigibius* may be at once separated from the other two genera dealt with in the present paper by the larger number of antennal articles and by their not having the posterior legs of males characterized by any special processes or lobes.

SIGIBIUS PURITANUS, sp. nov.

DIAGNOSIS.—Dorsal spines of third to eleventh pairs of legs, 0, 0, 0, 0, 1; ventral 0, 0, 0, 1, 1. Dorsal spines of first and second pairs, 0, 0, 0, 0, 1; ventral, 0, 0, 0, 0, 1.

Ocelli 3 to 6 in one or two series.

Prosternum near 1.57 times wider than long, Distance between chitinous spots $2\frac{1}{2}$ times the width at median incision; 3.86 times the dental line.

Coxal pores 2, 3, 3, 3-3, 4, 4, 3.

Claw of female gonopods wide bipartite or with obsolescent third tooth; the two main lobes short and rounded.

Body on average about 7.78 times longer than width of tenth plate.

Head a little longer than wide (42: 40 to 41: 40).

First dorsal plate about 1.7 times wider than long.

Brown to testaceous, often of dilute olivaceous caste; caudal dorsal plates and commonly 1st one darker, reddish brown. Head chestnut, light ferruginous in front of suture.

DESCRIPTION.— The dorsum is brown to testaceous, often of dilute olivaceous caste; caudal plates, and usually also the first one darker, reddish brown or chestnut, and one or more of the plates immediately caudad of the first one often with their posterior borders also of the darker color or the first plate also similarly darker only along its caudal border. Head chestnut in appearance, of a lighter ferruginous color in front of suture, behind which there is present a median black spot extending toward the caudal margin. Antennae mostly dark brown proximally, becoming lighter distad, the distal portion being mostly yellowish or sometimes of a rufous tinge. Prosternum dusky brown, the prehensorial feet lighter, rufous distally. Venter pale; the caudal plates darker, ferruginous to burnt brown; the anterior segments or entire venter suffused with purplish. Legs mostly pale, but the caudal pairs bright yellow or light orange excepting proximally, where they are brown.

Body strongly attenuated cephalad, about 7.8 times longer than width of tenth plate. Widths of head and of first, third, eighth, tenth, and twelfth plates to each other as 39: 38: 42: 50: 50.5: 50 in a female type; in male as 39: 37.5: 39: 46: 47: 46, the female being thus proportionately the broader caudad. Head subcordate, mostly a little longer than wide (42: 40 to 41: 40).

Antennae very short; articles mostly 25, very short; hairs moderately long, not very dense.

Eyes each consisting of from 3 to 6 ocelli arranged in one or two series:— *e. g.*, 1+2; 1+2, 1; 1+3, 2. Single ocellus largest.

Prosternum about 1.57 times wider than long. Distance between chitinous spots $2\frac{1}{2}$ times width at level of bottom of median incision; 3.86 times length of dental line. Median incision moderately wide,

sides straight and strongly converging. Teeth small and acute; the line of apices strongly recurving, the ectal tooth on each side being borne considerably farther forward than the mesal one. Spine inserted at base of outer tooth, scarcely stouter at base than the hairs, becoming slender and finely pointed distad.

First dorsal plate 1.7 times wider than long. Minor plates all with caudal corners obliquely somewhat excised; none with them at all produced.

Coxal pores 2, 3, 3, 3-3, 4, 4, 3; increasing in size distad on each coxa.

Spines of first and second pairs of legs, $\frac{0, 0, 0, 0, 1}{0, 0, 0, 0, 1}$; of third to eleventh, $\frac{0, 0, 0, 0, 1}{0, 0, 0, 1, 1}$; of twelfth, $\frac{0, 0, 0, 0, 0}{0, 0, 0, 1, 1}$; of thirteenth, $\frac{0, 0, 1, 0, 0}{0, 0, 1, 1, 1}$; of the penult, $\frac{0, 0, 1, 0, 0}{0, 1, 1, 1, 0}$; claws 2; of the anal also, $\frac{0, 0, 1, 0, 0}{0, 1, 1, 1, 0}$ claws 2.

Posterior legs in male, short, very moderately and uniformly enlarged.

Claw of female gonopods broad; the ectal lobe commonly almost or wholly obliterated, the other two lobes distinct, with the median usually considerably the larger, or sometimes the two lobes are nearly equal and bluntly rounded. Basal spines differing from those of species of *Tidabius* in being more slender and in being attenuated evenly from the very base to the acutely rounded apex, their being no distal acuminate portion sharply delimited from a basal. Inner edge of first article straight.

TYPE LOCALITY.—Massachusetts (Cambridge!).

LOCALITIES.—Massachusetts (Cambridge! Arlington Heights! Forest Hills! Waverley! Mattapan! Blue Hills! Wellesley! Hough's Neck!); Maine (Orono! Bangor! Hampden! Old Town!).

PLATE 1.

NAMPABIUS VIRGINIENSIS Chamberlin.

- Fig. 1. Prosternum.
- Fig. 2. Anterior border of prosternum more highly enlarged, showing teeth, ectal spines and form of mesal incision characteristic of the genus.
- Fig. 4. Penult leg of ♂ showing the characteristic process at distal end of tibia.
- Fig. 5. Process of penult leg more highly enlarged.

NAMPABIUS EMBIUS Chamberlin.

- Fig. 3. Left eye, showing characteristic form and arrangement of ocelli (single ocellus at the right of fig.).

NAMPABIUS FUNGIFEROPES Chamberlin.

- Fig. 6. Penult leg.
- Fig. 7. Process of penult leg more highly enlarged.

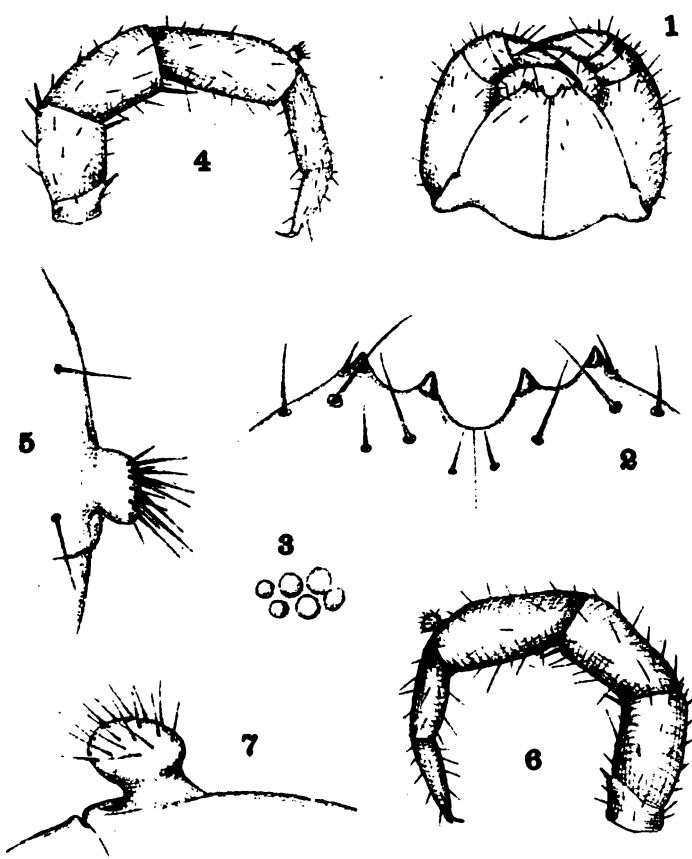


PLATE 2.

NAMPABIUS GEORGIANUS Chamberlin.

- Fig. 1. Penult leg, caudal (mesal) aspect.
- Fig. 2. Process of penult leg more highly enlarged.
- Fig. 3. Anal leg ectal aspect.

NAMPABIUS CAROLINENSIS Chamberlin.

- Fig. 4. Penult leg, caudal aspect.
- Fig. 5. Process of penult leg more highly enlarged.

NAMPABIUS VIRGINIENSIS Chamberlin. (See also Plate 1).

- Fig. 6. Left gonopod showing typical form of claw, basal spines, and proximal article.

NAMPABIUS EMBIUS Chamberlin.

- Fig. 7. Antenna.

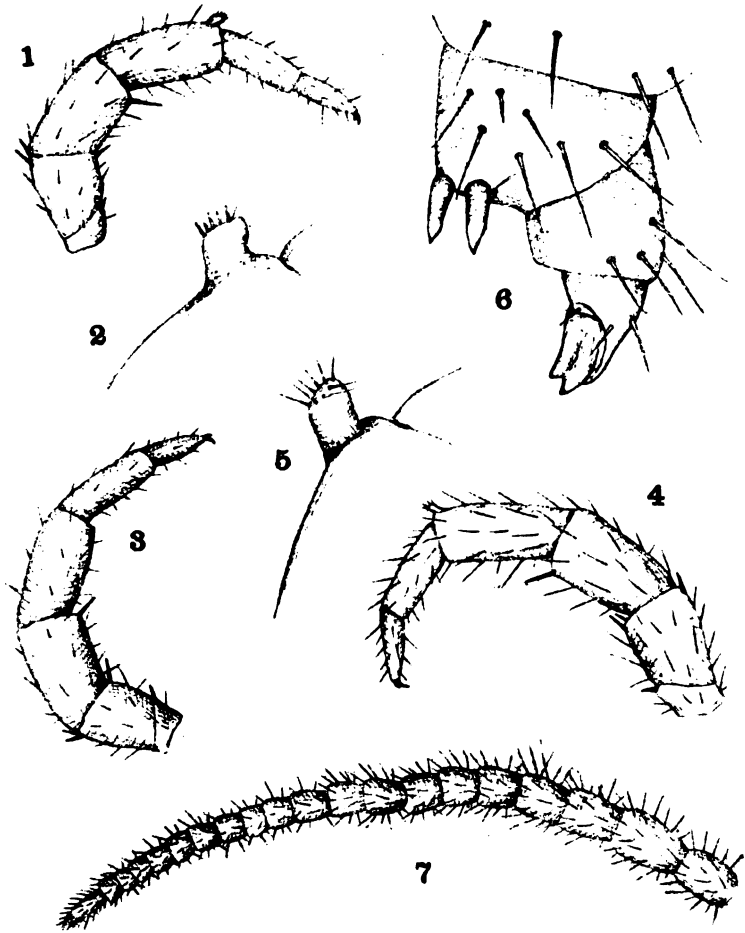


PLATE 3.

NAMPABIUS PARIENUS Chamberlin.

- Fig. 1. Penult leg, caudal aspect.
- Fig. 2. Process of penult leg more highly enlarged.

NAMPABIUS TENNESSEENSIS Chamberlin.

- Fig. 3. Penult leg.
- Fig. 4. Process of penult leg more highly enlarged.

NAMPABIUS INIMICUS Chamberlin.

- Fig. 5. Penult leg, caudal aspect.
- Fig. 6. Process of penult leg more highly enlarged.

NAMPABIUS VIRGINIENSIS Chamberlin. (See Plates 1, 2).

- Fig. 7. Head in outline, showing the more usual form in the genus.

NAMPABIUS GEORGIANUS Chamberlin. (See also Plate 2).

- Fig. 8. Head in outline.

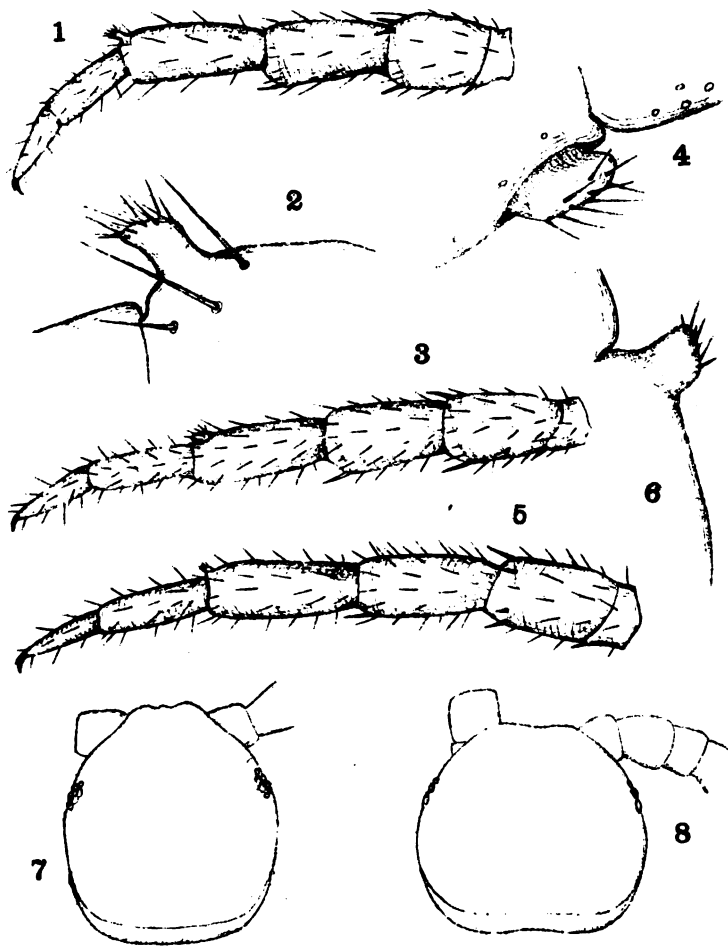


PLATE 4.

GARIBIUS MONTICOLENS Chamberlin.

- Fig. 1. Prosternum.
- Fig. 2. Anterior border of prosternum more highly enlarged, showing teeth, spines and form of median incision characteristic in the genus.
- Fig. 3. Antennae.
- Fig. 4. Left eye showing typical form and arrangement of ocelli (single ocellus at left).
- Fig. 6. Anal leg, mesal aspect, showing position and usual form of the tibial crest characteristic of males of the genus.
- Fig. 7. Tibial crest of anal leg, ectal view, more highly enlarged.

GARIBIUS CATAWBAE Chamberlin.

- Fig. 8. Anal leg, mesal view.
- Fig. 9. Tibial crest of anal leg, ectal view, more highly enlarged.

TIDABIUS TIVIUS Chamberlin.

- Fig. 5. Right gonopod, showing form of claw and of basal spines characteristic of the genus.

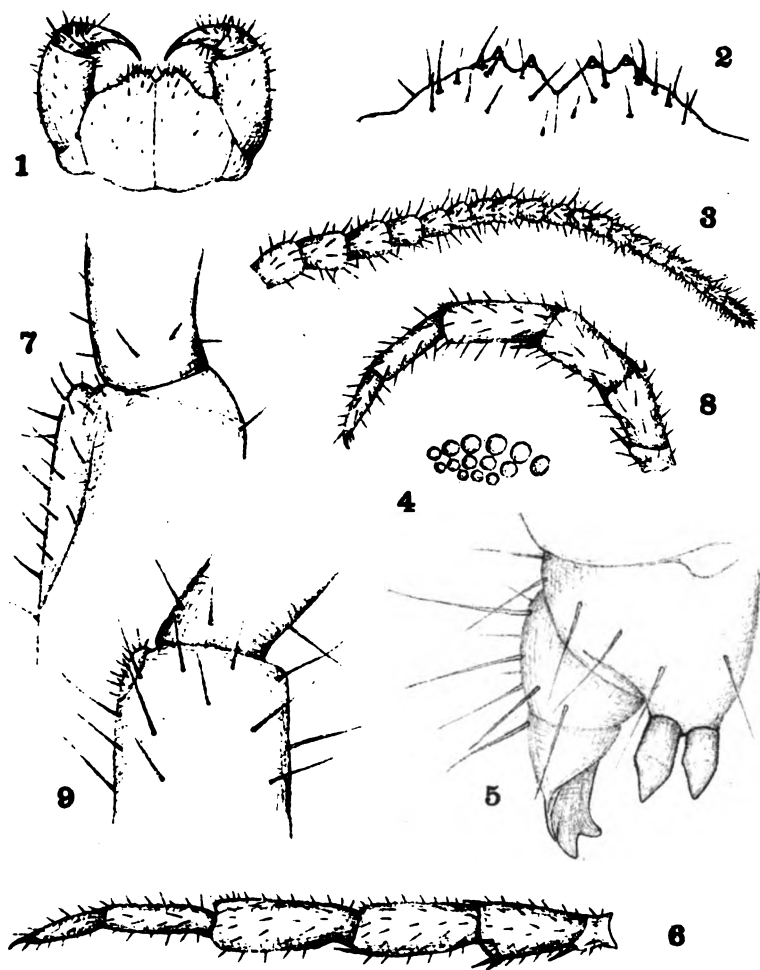


PLATE 5.

GARIBIUS MONTICOLENS Chamberlin. (See also Plate 4).

Fig. 1. Penult leg, caudal aspect.

TIDABIUS TIVIVS Chamberlin. (See also Plate 4).

Fig. 2. Prosternum.

Fig. 3. Anterior border of prosternum showing teeth spines and mesal incision, the latter of the form prevailing but not exclusively found in the genus.

Fig. 4. Anal leg of ♀, mesal aspect, showing the usual form for both sexes in the genus.

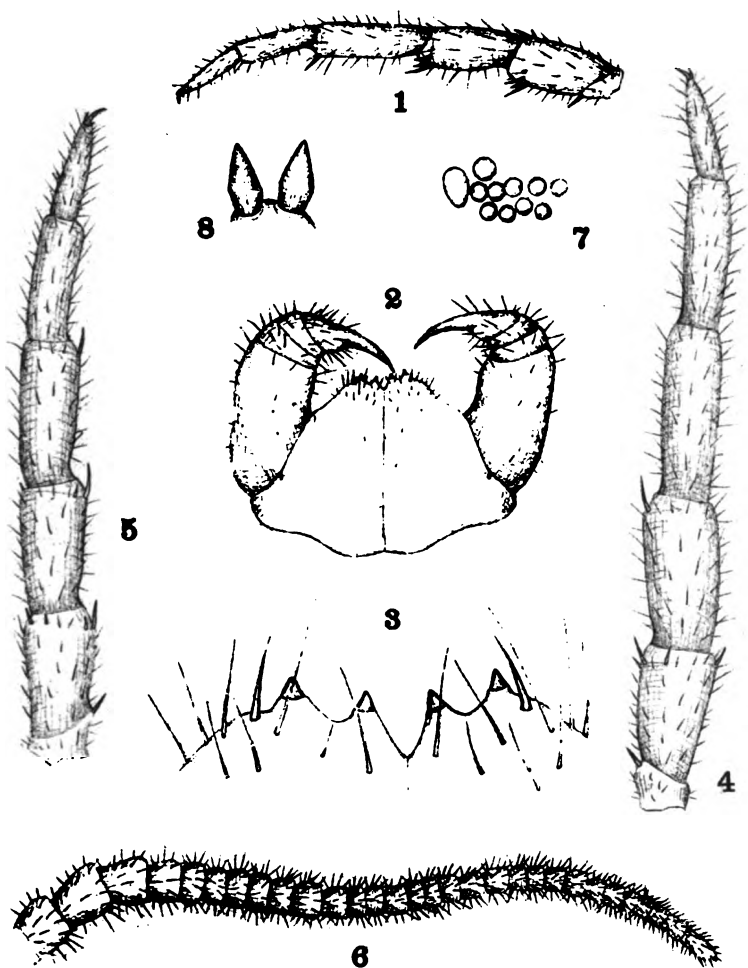
Fig. 5. Penult leg mesal (caudal) aspect.

Fig. 6. Antenna, representing the form typical in the genus.

Fig. 7. Right eye, showing characteristic form and arrangement of ocelli, (single ocellus at left of figure).

TIDABIUS OPIPHILUS Chamberlin.

Fig. 9. Basal spines of left gonopod of ♀.



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**THE STANFORD EXPEDITION TO BRAZIL, 1911, JOHN C.
BRANNER, DIRECTOR. THE CHILOPODA OF BRAZIL.**

BY RALPH V. CHAMBERLIN.

WITH SIX PLATES.

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No. 3.— *The Stanford Expedition to Brazil, 1911.* John C. Branner,
Director. *The Chilopoda of Brazil.*

BY RALPH V. CHAMBERLIN.

THE Brazilian chilopods upon a study of which the present paper is primarily a report, were collected for the most part by Mr. W. M. Mann as a member of the Stanford expedition to Brazil from June to September, 1911. As indicated hereafter, in the list by localities and under the particular species concerned, he was assisted in certain localities by Prof. Harold Heath and in others by Dr. Fred Baker. The collection was made almost wholly in parts of Brazil from which either few or no chilopods whatsoever have been previously recorded; and its study, in connection with that of some other material from the country, has brought about such a relatively material increase in the known fauna, that it has seemed advisable to give a complete review of the chilopods of Brazil. The Stanford Expedition collection has been purchased by the Museum of Comparative Zoölogy.

In Dr. Brölemann's *Catalogue des Myriopodes du Brésil* (São Paulo, 1909. *Catalogos de Fauna Brasileira*, 2, issued by the Museu Paulista), after the elimination of manifest synonyms and *nomina nuda*, there are mentioned sixteen genera and thirty-nine species of chilopods. The present paper lists seventy-one species under twenty-five genera. Of the additional forms, two families, three genera, and nineteen species have not been elsewhere recorded as occurring in Brazil, and of these one genus and seventeen species are described as new. In addition it has been deemed advisable to include descriptions of a new genus and three new species from the adjoining country of British Guiana, these having been studied in connection with the Brazilian material.

The following list shows the known geographical distribution of the species. From states not here listed no records have been published. The new forms, and those new to the Brazilian fauna, are starred. In addition to these, because of the new territory covered, nearly all of the records of species secured by the Expedition are new within Brazil and of interest and importance in throwing light upon distribution. The greater part of previously published records have been from the coastal states from Bahia southward, the most being from Bahia, Rio de Janeiro, and São Paulo. The States in which Mr. Mann and his associates worked are listed first and in order

below, each being preceded by a letter; while the particular localities within the states in which collecting was carried out are indicated by a preceding number.

A. RIO GRANDE DO NORTE.

1. *Natal*. (Mann. June).

* *Orphnaeus branneri*, sp. nov. *Scolopendra viridicornis* Newport. *Pselliophora nigrovittata* (Meinert). *Scolopendropsis calcaratus* (Pocock).

2. *Ceará-Mirim*. (Mann and Heath).

Orphnaeus brevilabiatus (Newport). *Trematophycus celeris* (Humbert and Saussure). *Scolopendropsis calcaratus* (Pocock).

B. CEARÁ.

3. *Ceará* (Mann).

Scolopendra viridicornis Newport.

C. PARAÍBA.

4. *Independencia* (Mann and Heath. Among the hills north of the town).

* *Schendylurus perditus*, sp. nov. * *Adenoschendyla parahybae*, sp. nov. *Orphnaeus brevilabiatus* (Newport). * *Cryptops heathi*, sp. nov. *Pselliophora nigrovittata* (Meinert).

5. *Parahyba*.

Scolopendra morsitans Linné.

D. PARÁ.

6. *Pará* (Mann and Baker. Chiefly in the suburb of Souza along trails through the forest. July).

Orphnaeus brevilabiatus (Newport). * *Schizonampa manni*, gen. et sp. nov. * *Newportia collaris* Kraepelin. * *Newportia paraensis*, sp. nov. *Scolopocryptops miersii* Newport. *Otostigmus goeldi* Brölemann. *Cupipes spinifer* Kraepelin. *Hemiscolopendra laevigata* Porat. *Scolopendra viridicornis* Newport. *Scolopendra morsitans* Linné.

Santarem.

Scolopendropsis bahiensis Brandt. *Scolopendra gigantea* Linné. *Scolopendra morsitans* Linné.

E. AMAZONAS.

7. *Manáos* (Mann and Baker. In and about a ruined church. August).

* *Schendylurus bakeri*, sp. nov. *Orphnaeus brevilabiatus* (Newport). *Notiphilides grandis* Brölemann. * *Mecistocephalus punctifrons* Newport. *Newportia amazonica* Brölemann. *Newportia bicegoi* Brölemann. *Newportia ernsti* Pocock. *Newportia longitarsis* (Newport). * *Ostostigmus amazonae*, sp. nov. * *Ostostigmus tidius*, sp. nov. *Trematophycus celeris* (Humbert and Saussure). *Cupipes unguilatis* Meinert. *Cupipes unguilatis mitis* Brölemann. * *Cupipes amazonae*, sp. nov. *Scolopendra morsitans* Linné. *Scolopendra viridicornis* Newport.

8. *Porto Velho* (Mann and Baker. September).

Newportia ernsti Pocock.

Obidos (Brazilian Guiana).

Scolopendra gigantea Linné.

Carsevenne or *Calçoene* River (Brazilian Guiana).

Adenoschendyla geayi Brölemann and Ribaut. *Thalthybius* (*Prionothalthybius*) *perrieri* Brölemann. *Ribautia bouvieri* Brölemann. *Newportia collaris* Kraepelin.

F. MATTO GROSSO.

9. *Madeira-Mamore R. R. Camp 39*. (284 km. from Porto Velho. Mann and Baker. September).

Newportia ernsti Pocock. * *Newportia longitarsis sylvae*, subsp. nov. * *Ostostigmus rex*, sp. nov. * *Ostostigmus casus*, sp. nov. *Trematophycus celeris* (Humbert and Saussure). * *Cupipes neglectus*, sp. nov. * *Scolopendra explorans*, sp. nov.

10. *Madeira-Mamore R. R. Camp 41*. (On the Rio Madeira 306 km. from Porto Velho. Mann and Baker. September).

* *Newportia longitarsis sylvae*, subsp. nov. *Scolopocryptops miersii* Newport. * *Ostostigmus suitus*, sp. nov. *Scolopendra angulata* Newport. *Pselliophora nigrovittata* (Meinert).

11. *Abuná*. (Nearly opposite mouth of Rio Abuná. Mann and Baker. September).

Trematophycus celeris (Humbert and Saussure).

Corumbá.

Aphilodon augustatus Silvestri.

Urucum.

Aphilodon angustatus Silvestri.

PERNAMBUCO.

*Villa Bella.**Scolopendra gigantea* Linné.*Pernambuco.**Cupipes unguatis* Meinert. *Scolopendra viridicornis* Newport.*Orphnaeus brevilabiatus* (Newport).*Rio Capivari.**Cryptops galathea* Meinert.

BAHIA.

*Bahia.**Otostigmus scabrieaudus* (Humbert and Saussure). *Trematophycus longipes* (Newport). *Scolopendropsis bahiensis* Brandt. *Scolopendropsis calcaratus* (Pocock). *Scolopendra viridicornis* Newport. *Scolopendra subspinipes* Leach.*Iguarassu.**Scolopendropsis bahiensis* Brandt.*Rio São Francisco.**Scolopendra viridicornis* Newport.*Santo Antonio da Barra.**Scolopendropsis calcaratus* (Pocock). *Pselliophora nigrovittata* (Meinert).

MINAS GERAES.

Otostigmus scabriceaudus (Humbert and Saussure). *Trematophycus brasiliensis* Kraepelin.*Lagoa Santa.*(?) *Geophilus* (*Schendylurus*?) *sublaevis* Meinert.

RIO DE JANEIRO.

*Campo Itatiaya.**Schendylurus luderwaldi* Brölemann and Ribaut.*Petropolis.**Cryptops iheringi* Brölemann.*Rio Espírito Santo.**Otostigmus scabriceaudus* (Humbert and Saussure). *Trematophycus brasiliensis* Kraepelin.*Rio de Janeiro.**Orphnaeus brevilabiatus* (Newport). (?) *Newportia aurantiaca* (Gervais). * *Mimops occidentalis*, sp. nov. *Otostigmus scabriceaudus* (Humbert and Saussure). *Cormocephalus aurantiipes* (Newport). *Scolopendra morsitans* Linné. *Scolopendra subspinipes* Leach.

SÃO PAULO.

Adenoschendyla imperfossa bolbonyx Brölemann and Ribaut.

Alto da Serra.

Cryptops iheringi Brölemann. *Otostigmus limbatus* (Meinert).

Otostigmus scabricaudus (Humbert and Saussure).

Belém.

Otostigmus caudatus Brölemann. *Hemiscolopendra laevigata* (Porat).

Fazenda Nova Nicaragua.

Schendylurus gounellei (Brölemann).

Itapetininga.

Otostigmus caudatus Brölemann.

Piquete.

Otostigmus scabricaudus (Humbert and Saussure). *Otostigmus tibialis* Brölemann.

Poco Grande.

Schendylurus paulista (Brölemann).

Santos.

Otostigmus limbatus Meinert. *Otostigmus tibialis* Brölemann.

São Paulo.

Otostigmus caudatus Brölemann. *Otostigmus tibialis* Brölemann.

PARANÁ.

Otocryptops ferrugineus macrodon Kraepelin. *Otostigmus tibialis* Brölemann.

Iguassú.

Mecophilus neotropicus Silvestri.

SANTA CATHERINA.

Blumenau.

Adenoschendyla plusiodonta (Attems).

RIO GRANDE DO SUL.

Cryptops iheringi Brölemann. *Cryptops galatheae* Meinert.

Porto Alegre.

Scolopocryptops miersii Newport.

São Laurenço.

Scolopocryptops miersii Newport.

BRAZIL (Without more definite locality).

? *Schendylurus brasilianus* (Silvestri). *Adenoschendyla imperfossa* (Brölemann). *Mecistauchenius micronyx* Brölemann. (?) *Newportia viridis* (Gervais). *Otocryptops ferrugineus* (Linné). *Otocryptops melanostomus* (Newport). *Trematophycus longipes* (Newport). *Scolopendra polymorpha* Wood. *Scolopendra alternans* Leach. *Cupipes brasiliensis* (Humbert and Saussure). *Lithobius forficatus* (Linné).

It seems scarcely necessary to point out the pronounced dominance of the Scolopendroidea in the Brazilian chilopod fauna and the practically complete absence of the Lithobiomorpha, so abundant in the Northern Hemisphere. It is probable, however, that a fair representation of the Henicopidae will later be found to occur. In the Geophiloidae the Schendylidae are manifestly dominant, the Oryidae coming next; while the Geophilidae proper are at present known with certainty to be represented by but two species, one of which is here first recorded and described.

SCOLOPENDROIDEA.

CRYPTOPIDAE.

Of this family, five genera (Cryptops, Mimops, heretofore known only from China, Newportia, Otocryptops, and Scolopocryptops) are represented in the Brazilian fauna, this being about half of the total number.

CRYPTOPS Leach.

Trans. Linn. Soc. London, 1814, 11, p. 384.

Key to Species.

- a. Tarsi of all legs distinctly biarticulate; last ventral plate with scattered dark spicules or spinous points which also cover the coxopleurae.....*C. iheringi* Brölemann.
- aa. Tarsi of only last two pairs of legs distinctly biarticulate; last ventral plate and coxopleurae not armed with spicules.
 - b. Second dorsal plate distinctly bisulcate; anterior margin of prosternum nearly straight.....*C. heathi*, sp. nov.
 - bb. Second dorsal plate without sulci; anterior margin of prosternum distinctly biarcuate.....*C. galathea* Meinert.

CRYPTOPS IHERINGI Brölemann.

Rev. Museu Paulista, 1901, 5, p. 42, pl. 1, fig. 6, 7; Kraepelin Revis. Scolop., 1903, p. 32, fig. 2; Brölemann, Cat. Myr. Brésil, 1909, p. 8.

Localities.—State of São Paulo: Alto da Serra (type loc.); State of Rio de Janeiro: Petropolis; State of Rio Grande do Sul.

CRYPTOPS GALATHEAE Meinert.

Vidensk medd. nat. foren. Kjøbenhavn, 1887, p. 140; Kraepelin, Revis. Scolop., 1903, p. 54.

Cryptops capivarae Pocock, Ann. mag. nat. hist., 1891, ser. 6, 8, p. 158; Brölemann, Cat. Myr. Brésil, 1909, p. 8.

Cryptops brasiliensis Attems, Mitt. Mus. Hamburg, 1900, 18, p. 112; Brölemann, Cat. Myr. Brésil, 1909, p. 8.

Localities.—State of Rio Grande do Sul (*brasiliensis* Attems); State of Matto Grosso: Rio Capivari (*capivarae* Pocock); Argentina: Montevideo (*galathea* Meinert).

CRYPTOPS HEATHI, sp. nov.

Color yellowish. Head a little darker than the body.

Head widest anteriorly, the sides converging caudad; caudal margin straight, meeting the edge of the first dorsal plate flush or scarcely overlapping the latter. Sulci not evident in middle and anterior portions; but rather faintly indicated caudad. Scarcely punctate.

Prosternum not punctate; with a median longitudinal furrow. Anterior margin nearly straight, being very slightly bowed caudad toward each end; bearing three bristles on each side. (Plate 1, fig. 2).

First dorsal plate with a distinct transverse cervical sulcus which is angularly bent caudad at the middle line, the plate being depressed at this angle. Two longitudinal sulci present, but these in the type are rather weak; forking cephalad, the inner branches meeting at an angle at the median line a little caudad of the cervical sulcus and the lateral lines each meeting the sulcus farther laterad (see Plate 1, fig. 1).

All dorsal plates from the second to twentieth longitudinally bisulcate. Plates not roughened; none of them bearing cornicles.

Last plate with a shallow median longitudinal furrow which is more evident toward the caudal end.

Ventral plates not punctate. Last ventral plate widely, semi-circularly, rounded caudad.

Coxopleurae subtruncate caudally, not at all extended. Pores few, small, not reaching the caudal edge by a large space.

Anterior legs not distinctly biarticulate. Anal legs with prefemur, femur, and, less distinctly, the tibia longitudinally furrowed dorsally. Prefemur with numerous spinules of the usual character over ventral and mesal surface; elsewhere with bristles; no longitudinal glabrous area on ventral side. Femur armed similarly to the prefemur but bearing in addition to the spinules a single stout tooth on the ventral surface toward the distal end. Tibia bearing ventrally toward the mesal edge a row of stout teeth; and the first tarsal joint bearing in corresponding location two similar teeth with an elevation distad of them as shown in Plate 1, fig. 3.

Length of type cir. 10 mm.

Locality.—State of Parahyba: Independencia! (Mann and Heath).

One specimen taken in the hills north of the town.

PARACRYPTOPS Pocock.

Ann. mag. nat. hist., 1891, ser. 6, 7, p. 227. Kraepelin, *Rev. Scolop.*, 1903, p. 59.

Previously this genus was known only from the East Indies, from where two species had been described. Another species has been recently described from India.

PARACRYPTOPS INEXPECTUS, sp. nov.

Color light lemon-yellow, darkest cephalad. Head darker than body, dilute orange-yellow excepting at anterior end where light yellow. Antennae and legs pale yellowish.

Cephalic plate overlapped by the first dorsal plate. Widest toward anterior end, from where the sides at first slightly and then more strongly converge to the caudal corners; anterior border of head subtriangular, notched at median line. On caudal portion with two short subparallel sulci; a pit-like depression a little distance from each lateral margin at middle of length; not punctate. Hair sparse.

Antennae composed of seventeen articles as usual. Densely clothed, with fine short hairs but these becoming longer and much sparser proximad.

Prosternum rather widely though but moderately depressed along the median longitudinal line. Anterior margin moderately extended cephalad, though less so than in *breviunguis*; margin each side of the narrow median incision broadly semicircular or with edges from rounded ectal ends to mesal incision substraight, the two sides meeting at an obtuse re-entrant angle; no distinct semilunar dental plates are present though there is a suggestion of the separation of the smooth, rounded, marginal portion suggestive of the condition in *weberi*.

First dorsal plate long; smooth; without furrows or with but very faint and short traces of a longitudinal pair toward anterior end. Submedian paired longitudinal sulci present on other dorsal plates from the second to the penult as are also the curved lateral sulci, the latter being sharply impressed excepting on the first few plates, where they are faint. Last dorsal plate with caudal produced border subtriangular, the median angle somewhat obtuse with the margin each side also forming a slight, very obtuse, angle near middle of its length. Plate with a very deep median longitudinal sulcus.

The ventral plates show a somewhat semicircular transverse impression in front of the level of the legs and a second transverse impression a little caudad of the legs, but not truly cruciform impression is indicated in the type. Last ventral plate with sides nearly straight, these converging to the semicircular caudal border.

Spiracles longitudinally elliptic.

Tarsi of anterior legs undivided. Legs clothed with sparse stiff bristles, these more spinescent on more caudal pairs. Prefemur of anal legs clothed with numerous long spines excepting dorsally and on median portion of ectal surface where they are replaced with fine hairs. Femur armed with similar or slightly more slender spines which are confined, however, more nearly to the strictly ventral surface; without teeth. Tibia without spines but bearing ventrally a longitudinal series of four teeth, these teeth curving caudad at distal ends. First tarsal joint with a single tooth at proximal end on ventral surface. Second tarsal joint without true teeth; but on ventral surface at proximal end it is extended into a conspicuous rounded process.

Length cir. 16 mm.

Locality.—British Guiana. One specimen taken at Washington, D. C., in pots of plants imported from British Guiana.

MIMOPS Kraepelin.

Revis. Scolop., 1903, p. 62.

This genus has heretofore been known from one species (*M. orientalis* Kraepelin) based upon a single specimen from China (Province Shensi). It was a matter of much surprise and interest, therefore, to find in the collection of the M. C. Z. two specimens of a distinct species but fully conforming to this genus in a vial with specimens of *Orphnaeus brevilabiatus* (Newport) from Rio de Janeiro. The specimens of *Orphnaeus* had been labeled *O. brasiliensis* by Meinert, who seems to have overlooked or to have failed to examine critically the smaller specimens of *Mimops*. It is, of course, quite possible that the specimens were introduced to Rio de Janeiro on ships from the East; but this must remain for the present uncertain.

MIMOPS OCCIDENTALIS, sp. nov.

The color of the types appears to have been yellowish; but because of long preservation the original color of the specimens cannot be satisfactorily ascertained.

Cephalic plate wider than long, nearly in ratio 12:11. A little overlapping the first dorsal plate. Widest anteriorly, with sides converging caudad; caudal margin mesally a little incurved; the anterior margin incised between bases of antennae. Longitudinally depressed in caudal region each side of middle. (Plate 1, fig. 4).

Antennae composed of seventeen or eighteen articles, which in the proximal half are as wide as or wider than long, but distad become longer than wide.

Anterior margin of prosternum nearly straight being but very weakly widely convex; bearing two moderately high, distally rounded, dental plates separated by a median space or incision which is rounded at bottom and is deeper than in *orientalis* Kraepelin. All joints of prehensorial feet unarmed. Claw short and stout and but little curved. (Plate 1, fig. 5).

First dorsal plate with a transverse sulcus a little caudad of margin of head, this sulcus bending caudad at middle region. Also with a longitudinal furrow each side of the middle extending cephalad from the caudal margin and uniting at an angle with its fellow near the middle of the plate, from where they continue as a single median furrow to the transverse sulcus (Plate 1, fig. 4). Other dorsal plates bi-

sulcate. The sulci of the second and third as well as of the others entirely crossing the plate. A ridge-like elevation or keel between the sulci. Plates longitudinally depressed on each side between the sulcus and the lateral margin. (Plate 1, fig. 6). Last dorsal plate margined. Bowed out caudad with the mesal part truncate, the margin on each side of truncation slightly incurved and extending obliquely to the lateral margin. The two longitudinal sulci also evident on this plate excepting at caudal end.

Second to nineteenth ventral plates longitudinally bisulcate. First plate with a median longitudinal furrow. Especially the more posterior plates longitudinally broadly depressed each side of the middle. Last ventral plate strongly narrowed caudad, though less strongly so than in *orientalis*. Caudal margin straight or but slightly excurved; corners rounded.

Coxopleurae extended caudad in a conical process which is stouter and less cylindric than in *orientalis*. Pores small and numerous. (Plate 1, fig. 7).

Tarsi of all legs distinctly biarticulate. Anal legs of form very similar to that of *orientalis*. Prefemur with a low dorsal elevation at distal end. Claw much shorter than tarsus.

Length 10.5 mm.

Locality.—State of Rio de Janeiro: Rio de Janeiro. Nathaniel Thayer expedition. 1864. M. C. Z.

Because of long preservation the two specimens are bleached and almost wholly bereft of hairs and spinules; accordingly, no attempt is made to describe their presence and characteristics. *Mimops orientalis* Kraepelin, the other species of the genus, is from Shensi, China. It is a much larger form and differs in numerous structural details from the present species.

OTOCRYPTOPS Haase.

Abhandl. Mus. Dresden, 1887, 5, p. 96.

Scolopocryptops Newport (in part), Trans. Linn. soc. London, 1844, 19, p. 405.

Meinert (ad part. max.), Proc. Amer. philos. soc., 1886, 23, p. 179.

Otocryptops Kraepelin, Revis. Scolop. 1903, p. 68. Verhoeff, Bronn's Thierreich, 1907, 5, p. 255.

Key to species.

- a. Twentieth, and often also the twenty-first, legs with a spine both on tibia and on tarsus; prosternum with anterior margin

bearing two to four more or less distinct teeth or dentiform elevations.

b. Basal tooth of first joint of prehensors small or but moderate in size, being at base from one eighth to one tenth as thick as the joint.....*O. ferrugineus* (Linné).

bb. Basal tooth of first joint of prehensors large, being at base nearly one fourth as wide as the joint.

O. ferrugineus macrodon Kraepelin.

aa. Twentieth to twenty-third pairs of legs always lacking tibial and tarsal spines; prosternal margin smooth, without trace of teeth.

O. melanostomus (Newport).

OTOCRYPTOPS FERRUGINEUS (Linné).

Scolopendra ferruginea Linné, Syst. nat. ed., 12, 1767, 6, p. 1063.

Scolopocryptops ferruginea Newport, Trans. Linn. soc. London, 1844, 19, p. 406.

Scolopocryptops rufa Gervais, Insect. Aptères, 1847, 4, p. 297.

Scolopocryptops mexicana Humbert et Saussure (non Saussure, 1860), Rev. mag. zool., 1869, p. 158.

Scolopocryptops sexspinosus Porat (non Say), Bih. Svensk. vet. akad. Handl., 1876, 4, no. 7, p. 26. Kohlrausch (in part), Arch. naturg., 1881, 47, 1, p. 54.

Scolopocryptops antillarum Marsh, Trans. Ent. soc. London, 1878, p. 37.

Scolopocryptops miersii Meinert (ad part max), Proc. Amer. philos. soc., 1886, 23, p. 181.¹

Scolopocryptops bisulca Karsch, Abhandl. Naturw. ver. Bremen, 1887, 9, p. 66.

Scolopocryptops strigilis Karsch, Ibid.

Scolopocryptops meinerti Pocock, Ann. mag. nat. hist., 1888, ser. 6, 2, p. 474.

Otocryptops ferrugineus Kraepelin, Revis. Scolop., 1903, p. 72.

Otocryptops sexspinosus Brölemann (non Say, the Brazilian record), Cat. Myr. Brésil, 1909, p. 11.

Locality.— "Brazil."

This is a widely distributed species in Mexico, Jamaica, Haiti, and the Antilles generally, Central America, Ecuador, and Peru.

¹ Of the specimens in the M. C. Z. labeled by Meinert as *S. miersii* and reported upon in the paper cited above, one specimen, from Martinique, is the true *S. miersii* Newport, the others being *O. ferrugineus*.

OTOCRYPTOPS FERRUGINEUS MACRODON Kraepelin.

Revis. Scolop., 1903, p. 74.

Locality.— State of Paraná: Paraná (sec. Kraepelin).

Separated by Kraepelin from the species on the basis of the larger tooth on the first joint of the prehensors as indicated in the key above.

OTOCRYPTOPS MELANOSTOMUS (Newport).

Scolopocryptops melanostoma Newport, Trans. Linn. soc. London, 1844, **19**, p. 406; Gervais, Insect. Aptères, 1847, **4**, p. 298.

Scolopocryptops megalcephalus Kohlrausch, Archiv. naturg., 1881, **47**, 1, p. 57.

Scolopocryptops luzonicus Kohlrausch, Ibid., p. 58.

Scolopocryptops boholiensis Kohlrausch, Ibid., p. 58.

Scolopocryptops geophilicornis Tömösvary, Termes. füzetek, 1885, **9**, p. 65.

Otocryptops luzonicus Haase, Abhandl. Mus. Dresden, 1887, **5**, p. 98.

Otocryptops luzonicus australis Haase, Ibid., p. 98.

Otocryptops longiceps Pocock, Ann. mag. nat. hist., 1890, ser. 6, **8**, p. 160.

Otocryptops melanostoma Pocock, Journ. Linn. soc. London, 1891, **24**, p. 464;

Brölemann, Ann. Soc. ent. France, 1898, **67**, p. 250; Kraepelin, Revis. Scolop., 1903, p. 74, fig. 33, 34.

Otocryptops aculeatus Attems, Abhandl. Senckenb. gesellsch., 1897, **23**, p. 478.

Locality.— Brazil (recorded as *O. longiceps* by Pocock).

Also known from Argentina and Venezuela and Porto Rico, St. Vincent, etc., and occurring widely in the East Indies as well.

SCOLOPOCRYPTOPS Newport.

Newport (in part), Trans. Linn. soc. London, 1844, **19**, p. 405; Kraepelin,

Revis. Scolop., 1903, p. 76; Verhoeff, Bronn's Thierreich, 1907, **5**, p. 255.

The following is the only species known from the Western Hemisphere.

SCOLOPOCRYPTOPS MIERSII Newport.

Trans. Linn. soc. London, 1844, **19**, p. 405; Meinert (in part min.), Proc. Amer.

philos. soc., 1886, **23**, p. 181; Pocock, Journ. Linn. soc. London, 1893, **24**,

p. 146; Silvestri, Ann. Mus. civ. stor. nat. Genova, 1895, ser. 2, **14**, p. 24;

Brölemann, Ann. Soc. ent. France, 1898, **67**, p. 250; Rev. Museu Paulista,

1901, **5**, p. 42; Kraepelin, Revis. Scolop., 1903, p. 77; Brölemann, Cat.

Myr. Brésil, 1909, p. 33.

Localities.— State of Matto Grosso: Madeira-Mamore R. R. camp 41 on the Rio Madeira! (W. M. Mann); State of Para: Para, suburb of Souza! (Mann and Baker); State of Rio Grande do Sul: Porto Alegre, São Laurenço.

This species is common from the southern United States southward through Mexico and Central America to Venezuela, Guiana, and Brazil.

NEWPORTIA Gervais.

Insect. Aptères, 1847, 4, p. 298; Kraepelin, Revis. Scolop., 1903, p. 76; Verhoeff, Bronn's Thierreich, 1907, 5, p. 251.

Newportia + *Scolopendrides*, Saussure, Rev. mag. zool. 1869, ser. 2, 21, p. 158.

This genus, peculiar to tropical and subtropical America, is represented in Brazil by seven known species, of which four have been previously recorded. Of the three here first listed from Brazil, two are described as new. In addition, *Scolopocryptops aurantiaca* and *S. viridis* Gervais (Insect. Aptères, 4) are probably based upon members of the present genus; but there is nothing in the original descriptions to make precise identification possible, and the names must be dropped until the types are examined, if they now be in existence.

Key to Species.

- a. Distal division of tarsus of anal legs indistinctly many ringed, the divisions not clearly separated or numerable; tibia of legs, excepting the last three pairs, armed both laterally and ventrally with a stout spine; tarsus of these legs also with a stout ventral spine; spiracles very small. (*Scolopendrides* Saussure).
 - b. Anal leg terminating in a well-developed claw.

N. amazonica Brölemann.
 - bb. Anal leg clawless.
 - c. Paired longitudinal sulci of head crossed near caudal ends with a fine and distinct transverse sulcus; sulci of second dorsal plate evident from anterior margin caudad to or past the middle of plate. *N. ernsti* Pocock.
 - cc. Paired longitudinal sulci of head not crossed near base by any such transverse sulcus; sulci of second dorsal plate not evident on anterior half, being present only as short lines at caudal border which bifurcate into a very short mesal branch and an ectal one that runs almost directly ectad to the lateral margin. *N. paraensis*, sp. nov.

- aa. Distal division of tarsus of anal legs composed of a limited number of articles clearly separated from each other; tibia of anterior pairs of legs with only a lateral spine; tarsi of these legs without any ventral spine; spiracles large and distinct.

(*Newportia sens. str.*).

- b. First dorsal plate with a simple, semicircular or nearly semicircular transverse cervical sulcus; its paired longitudinal sulcus simple and undivided; no median, pit-like, depression caudad of median sulcus.

- c. Prefemur of anal legs on dorsomesal surface with two rows of from seven to ten spinules.

N. longitarsis (Newport).

- cc. Prefemur of anal legs without any spinules proper additional to the large ventral spines.

N. longitarsis sylvae, subsp. nov.

- bb. First dorsal plate with the transverse cervical sulcus bent angularly caudad at middle and with a pit-like, median, depression caudad of its apex; paired longitudinal sulci bifurcating cephalad, the inner branches meeting in the depression and the lateral extending cephalo-ectad to the transverse sulcus, the branches together forming a more or less w-shaped outline.

- c. First joint of tarsus of anal legs clavately thickened distad and with corner drawn out at side into a pointed angle; part of first plate in front of transverse sulcus about as long as that caudad of it.

N. collaris Kraepelin.

- cc. First joint of tarsus of anal legs not thus clavately thickened distad; cervical sulcus considerably cephalad of middle of first plate. *N. bicegoi* Brölemann.

NEWPORTIA AMAZONICA Brölemann.

Rev. Museu Paulista, 1903, 6, p. 69, pl. 11, fig. 3, 4; Brölemann, Cat. Myr. Brésil, 1909, p. 9.

Locality.—State of Amazonas: Manáos! (Mann and Baker. This is also the type locality).

Kraepelin says (Revis. Scolop., p. 85) with reference to this species: —“*S. amazonica* Bröl. scheint sich von der vorsthenden Art [N.

ernsti' vornehmlich durch den Besitz eines winzigen Klaue am Ende der Tarsengeißel zu unterscheiden. Ich glaube kaum das es sich hier um eine artliche Verschiedenheit, sondern um eine individuelle Rückschlagsbildung handelt, die im interessanter Weise die Herkunft der Newportien aus Formen mit Klauen tragenden Analbeinen demonstriert."

But this author is clearly mistaken in regarding the claw thus as an individual atavistic variation, inasmuch as a normal and well-developed claw is present in all the specimens secured in the type locality by Mr. Mann (Plate 2, fig. 4). In *Newportia ernsti*, occasionally a very small and wholly straight chitinous point may occur at the tip of the tarsus (Plate 2, fig. 5), but apparently never a true claw or anything that might be regarded as properly transitional to the condition in *amazonica*. The spining of the prefemur, etc., is also constantly different, there being in all the specimens examined but four ventral spines instead of six, these being also relatively considerably larger.

NEWPORTIA ERNSTI Pocock.

Ann. mag. nat. Hist. 1891, ser. 6, 8, p. 161; Kraepelin, Revis. Scolop., 1903, p. 85, fig. 38, 39; Brölemann, Cat. Myr. Brésil, 1909, p. 10.

Localities.—State of Amazonas: Manáos!, Porto Velho! (Mann and Baker). State of Matto Grosso: Madeira-Mamore R. R. camp 39 on the Rio Madeira (W. M. Mann).

Numerous specimens were secured at Manáos and several at each of the other two places indicated. Closely related to the preceding species.

NEWPORTIA PARAENSIS, sp. nov.

Color yellow of a reddish tinge, with the most caudal plates darker.

Head with prosternum and prehensors and the first dorsal plate reddish brown. Antennae yellow, darkest proximally. Head with punctations distinct and numerous, not fine, more sparse in frontal region, particularly in a transverse band across its caudal portion. Two median sulci distinct forward to middle of plate and not crossed by any transverse sulcus; a median longitudinal furrow from anterior margin a little distance caudad. Caudal margin widely convex, a little incurved right of middle portion of sides straight and parallel; caudal corners widely rounded.

Antennae short, composed of sixteen (mostly) or seventeen articles. None of the articles glabrous but the first two more sparsely clothed with hair than the others, the density increasing from the second to the fourth and thereafter essentially uniform.

Anterior margin of prosternum considerably more elevated ectally than mesally; bearing two wide but very short dental plates which are weakly convex.

First dorsal plate with a strictly semicircular cervical sulcus which is entirely free from the head and at its middle nearly one third the distance from the head to the caudal margin of the plate. Median longitudinal sulci distinct, subparallel, crossing the transverse sulcus and attaining the front margin.

Second dorsal plate with the median longitudinal sulci appearing as very short lines at caudal border, each line being continuous with a sulcus extending a little cephalad of directly ectad to the lateral margin and with a very short line extending mesocephalad. The median longitudinal sulci are complete on the other plates. The third plate shows a rather wide and shallow median longitudinal furrow. Subsequent plates with a distinct narrow median longitudinal keel set off by two deep furrows. Plates from fifth caudad with a distinct longitudinal furrow on each side with also less distinct indications of similar ones on the third and fourth.

Last dorsal plate bowed considerably caudad, the protruding mesal portion truncate. Without a median sulcus or with but weak trace of such toward caudal end.

Ventral plates from the second to the penult with a strongly marked median longitudinal furrow which is continuous from the anterior margin to a little in front of the caudal border. Lateral sulci extending from anterior margin to caudal portion of plate, converging with each lateral margin. Not distinctly punctate.

Last ventral plate considerably wider than long; narrowed caudad; posterior corners well rounded; caudal margin widely though but moderately convex, slightly crenately notched each side of the middle.

Spiracles moderate; mostly elliptical, being obliquely or rather more dorsoventrally compressed.

Coxopleural processes long and straight; ending in a single slender and acute spine; process with but scattered short hairs.

Prefemur of anal legs armed ventrally with a series of six large spines and, in addition, on mesal surface with about eighteen spinules arranged in four longitudinal series. Femur armed ventrally with two spinules on proximal half and in a longitudinal line. Other joints

unarmed. Tibia with rather sparse and moderately long hairs. Joints of tarsus with more numerous similar hairs. Second division of tarsus indistinctly segmented. First joint or division of tarsus considerably thicker than the second division and more slender than the tibia; half or a little more than half the length of the latter (17:32.) (Plate 2, fig. 3).

Length of largest specimen 28 mm.

Locality.— State of Pará: Pará, suburb of Souza! (Mann and Baker). Three individuals, two adult and one immature, were secured.

NEWPORTIA LONGITARSIS (Newport).

Scolopocryptops longitarsis Newport, Trans. Linn. soc. London, 1844, 19, p. 407, pl. 40, fig. 10.

Newportia longitarsis Gervais, Insect. Aptères, 1847, 4, p. 298; Humbert et Saussure, Rev. mag. zool., 1869, ser. 2, (31), p. 159; Miss. scient. Mex., 1872, p. 138; Pocock, Journ. Linn. soc. London, 1893, 24, p. 416; Brölemann, Ann. Soc. ent. France, 1903, 67, p. 251; Kraepelin, Revis. Scolop., 1903, p. 86; Brölemann, Cat. Myr. Brésil, 1909, p. 10.

Locality.— State of Amazonas: Manáos. (sc. Brölemann); Colombia; Central America, etc.

NEWPORTIA LONGITARSIS SYLVAE, subsp. nov.

General color ochre-yellow, most of the dorsal plates being darker, more reddish, along caudal borders. Head darker, of a more ferruginous tinge. Antennae and legs yellowish.

Head deeply and regularly, but not densely, punctate. The paired submedian sulci present only as short impressions at caudal border; a short, wider, transverse furrow, in front of their anterior ends. A rather fine median longitudinal furrow at anterior end.

Antennae composed of from fifteen to seventeen articles, there being in one type specimen fifteen in the right antenna and sixteen in the left, one of those in the latter being, however, of double length and apparently representative of two normal articles. None of the articles shining or glabrous; but the hairs of the first two distinctly more sparse, those of third and fourth more dense, but only the fifth and subsequent articles fully clothed in the usual manner.

Prosternum with anterior margin nearly straight, being a little more

elevated at each ectal end than at middle where it is very slightly notched; edge well chitinized but without true dental plates. Subsparsely punctate.

Cervical furrow of first dorsal plate strictly semicircular, distinctly exposed excepting laterally. Two median longitudinal sulci distinct; parallel excepting toward anterior ends where they diverge somewhat and finally meet the transverse sulcus; not at all branched anteriorly; but near caudal end each is joined by a strictly transverse sulcus which extends out toward lateral margin. Plate semicircularly depressed transversely in middle region near cervical sulcus (Plate 2, fig. 1). Second dorsal plate with paired sulci extending entirely across length; converging cephalad and near anterior end united with a network of very fine anastomosing transverse lines or sulci (Plate 2, fig. 1). Other dorsal plates to and including the twenty-second with two distinct and subparallel longitudinal sulci across entire length. The third plate with a fine but distinct straight sulcus running from anterior end of each longitudinal sulcus obliquely caudoectad to the lateral margin (Plate 2, fig. 1). Plates from the fifth to the twenty-second inclusive with a longitudinal furrow between each longitudinal submedian sulcus and the lateral margin. Last dorsal plate without a median longitudinal furrow. Caudally bowed out, with the extended mesal portion truncate.

Ventral plates with the usual median longitudinal furrow joining the distinct transverse sulcus across the caudal portion of plate but not extending across the anterior portion. Also with a distinct abbreviated longitudinal sulcus on each side convergent with lateral margin. Plates sparsely punctate. Last ventral plate considerably narrowed caudad. Caudal margin moderately incurved at middle. Wider than long in about ratio 34:29.

Spiracles moderately large; mostly narrower ventrad, roundly subtriangular.

Coxopleurae of twenty-third segment with caudal processes rather short, ending in a single spine-pore area extensive.

Tarsi of anterior legs not distinctly divided. Legs clothed sparsely with stiff bristles, but with no spinules. Tibiae of anterior pairs armed laterally at distal end with a stout spine. Prefemur of anal legs armed ventrally with a row of four stout spines which increase regularly in size distad; on mesal surface with about four irregular series of small bristles and with a similar series on dorsal side near mesal edge and also similar series over ectal surface, also a few scattered longer bristles present, but no true spinules present. Femur

with a stout spine on mesal surface near the proximal end and toward the ventral surface; otherwise unarmed. Other joints, so far as ascertainable from types, bearing rather scattered and short hairs, excepting the tarsi on which they are longer and more dense. Tibia broadly constricted toward each end. First article of tarsus a little more than half as long as the tibia (ratio cir. 17:30); of same thickness as the immediately succeeding articles; the latter distinct and clearly separated from each other. (Plate 2, fig. 2).

Length cir. 36 mm.

Localities.— State of Matto Grosso: Madeira-Mamore R. R. camps 39 and 41 on the Rio Madeira! (W. M. Mann). One specimen from each locality.

NEWPORTIA COLLARIS Kraepelin.

Revis. Scolop. 1903, p. 90.

Localities.— State of Pará: Pará, suburb of Souza! (Mann and Baker); State of Amazonas: Lower Carsevenne, Brazilian Guiana.

This second locality is the type locality and the two are the only ones recorded for the species.

NEWPORTIA BICEGOI Brölemann.

Rev. Museu Paulista, 1903, 6, p. 67, pl. I, fig. 1; Kraepelin, Revis. Scolop., 1903, p. 93.

Locality.— State of Amazonas: Manáos.

OTOSTIGMIDAE.

This tropical and subtropical family is known from Australia, Asia, Africa, and the warmer parts of America. It is represented in the known fauna of Brazil by two genera, *Otostigmus*, the large typical genus, and *Trematophycus*.

OTOSTIGMUS Porat.

Bih. Svensk. vet. akad. Handl., 1876, 4, no. 7, p. 18; Meinert, Vid. Medd. nat. foren. Kjøbenhavn, 1884, p. 118; Proc. Amer. philos. soc., 1886, 23, p. 183; Pocock, Biol. Centr. Amer. Chilopoda, 1895, p. 25; Kraepelin, Revis. Scolop., 1903, p. 97; Verhoeff, Bronn's Thierreich, 1907, 5, p. 254,

Branchiotrema Kohlrausch, Journ. Mus. Godef., 1878, p. 70; Archiv. naturg., 1881, 47, 1, p. 70.

Parotostigma Pocock, Biol. Centr. Amer. Chilopoda, 1895, p. 25.

Of this genus nine species are at present known from Brazil, five of these being here described as new.

Key to Species.

- a. Tarsal spines wholly absent or, rarely, a few of the legs with a much reduced spine. *O. limbatus* (Meinert).
- aa. Tarsal spines present and distinct.
 - b. Dorsal plates of caudal half of body distinctly scabrous, bearing rows of fine elevated spinous points.
 - c. Last ventral plate without distinct median sulcus; only the two proximal articles of the antennae glabrous; with five rather small keels or keel-like elevated lines on dorsal plates of caudal portion of the body. . . *O. casus*, sp. nov.
 - cc. Last ventral plate with a distinct median longitudinal sulcus; two and a fourth or two and a half proximal articles of antennae glabrous; only a single, flat, median keel present on dorsal plates.
 - d. Twentieth legs without a tarsal spine; only one tooth on each dental plate distinct, the others being completely fused; head and first dorsal plate abruptly different in color from the other plates, brownish.
 - O. rex*, sp. nov.
 - dd. Twentieth legs with a tarsal spine; each dental plate with four distinct teeth; head and first dorsal plate not abruptly different in color from the other plates, olivaceous. . . . *O. scabricaudus* Humbert et Saussure.
 - bb. All dorsal plates smooth, those of the caudal half of the body not distinctly scabrous.
 - c. First eighteen or nineteen pairs of legs with two tarsal spines.
 - d. First eighteen pairs of legs with two tarsal spines; twentieth legs with a tarsal spine; dorsal plates with a conspicuously elevated double keel each side of middle, the dorsal sulcus of each side lying between the halves of this keel. *O. tidius*, sp. nov.

- dd. First nineteen pairs of legs with two tarsal spines; twentieth legs with no tarsal spine; dorsal plates without such conspicuous keels or ridges.

O. goeldi Brölemann.

- cc. Legs of only the first two to the first six pairs with two tarsal spines or all with but a single tarsal spine.

- d. Last dorsal plate in the male ending in a process as long as the plate proper, in the female caudally acutely angular or at least rectangular; ventral plates wholly without furrows or pits. *O. caudatus* Brölemann.

- dd. Last dorsal plate in both sexes simply bowed out caudad, not acutely angular; ventral plates with distinct depressions or pits.

- e. Ventral plates from the second to the twentieth with distinct sulci reaching to beginning of caudal third or fourth of length; first six pairs of legs with two tarsal spines; only the first two articles of antennae glabrous. *O. amazonae*, sp. nov.

- ee. Sulci of ventral plates indicated only as short traces at the anterior border; only the first two pairs of legs with two tarsal spines; first three articles of antennae glabrous.

O. tibialis Brölemann.

OTOSTIGMUS LIMBATUS Meinert.

Vid. Medd. nat. foren. Kjøbenhavn, 1884, p. 120; Karsch, Berl. ent. zeitschr., 1888, **32**, p. 31; Silvestri, Ann. Mus. civ. stor. nat. Genova, 1895, ser. 2, **14**, p. 766; Boll. Mus. zool. anat. comp. R. univ. Torino, 1895, **10**, p. 23; Brölemann, Rev. Museu Paulista, 1901, **5**, p. 37; Kraepelin, Revis. Scolop., 1903, p. 130; Cat. Myr. Brésil, 1909, p. 13.

Localities.—“Brazil” (sec. Meinert; spec. Mus. Copenhagen); State of São Paulo: Alto da Serra, Santos.

This species is also known from Paraguay, from where two of Meinert's typical specimens came, and from Argentina (Buenos Aires).

OTOSTIGMUS AMAZONAE, sp. nov.

Bluish green to olive-brown; with a fine median longitudinal pale line. Head distinctly darker, deeper green. Antennae bluish green.

proximally, paler distad. Legs more pigmented distally than proximally; the posterior pairs green or bluish green excepting toward base.

Head shining; punctae weak and more or less scattered. The usual two longitudinal furrows of the caudal portion, these being shallow.

Antennae composed of seventeen articles of which only the first two are glabrous.

Prosternal teeth 4-4 of which the outermost on each side is more remote and is separated by a deeper interval than the others are from each other; innermost tooth on each side smallest, the two intermediate ones of nearly equal size.

Dorsal plates from the fourth segment on distinctly bisulcate. Only the twenty-first plate truly margined but the others of the posterior half of the body especially, with submarginal longitudinal furrows or depressions which simulate true margination. Plates, wholly smooth and with no indication on any of a median keel. Last plate more or less angularly produced, the margin bent in on each side of the middle (Plate 3, fig. 2); with no developed sulci or pits, the plate somewhat longitudinally elevated along the median line and faintly depressed or furrowed along the middle of this.

Ventral plates from second to twentieth with two longitudinal sulci extending to caudal third or fourth of length where each at its end is more deeply impressed. A short pit-like, median, depression in front of the caudal margin and a less pronounced median depression farther cephalad. Last ventral plate convex. Strongly narrowed caudad; caudal margin mesally excised. With a weak median sulcus and also on each side a faint fine sulcus from anterior margin to near middle (Plate 3, fig. 1).

Coxopleurae not produced, being caudally simply rounded; unarmed.

First six pairs of legs with two tarsal spines; the seventh to nineteenth with one; twentieth legs with none. Anal legs wholly unspined, being smooth throughout.

Length 23 and 32 mm.

Locality.—State of Amazonas: Manáos! (Mann and Baker).

Two specimens were secured.

This species seems to be related to *O. limbatus* Meinert, but is very easily separated through the differences in the spining of the tarsi as indicated in the key.

Otostigmus suitus, sp. nov.

Color olive-green with some of the dorsal plates appearing darker along the caudal margin. Antennae more brownish excepting at base.

Head distinctly punctate. Marked in front of caudal margin with two short longitudinal furrows.

Antennae seventeen jointed.¹ First two or two and a half articles glabrous (the third in type partially rubbed so that precise extent of glabrous condition is uncertain); other articles clothed densely with the usual short brown hairs.

Dorsal plates from the fourth, inclusive, caudad with two distinct median sulci; a longitudinal depression or furrow on each side, but true margination present only on the twenty-first plate, plates mostly depressed between median sulci and with a weakly developed median keel indicated on plates from the third caudad; the surface and edges of plates wholly smooth. Last dorsal plate with posterior margin bowed moderately caudad. A median pit-like depression in front of caudal margin and a keel in front of this as on the other plates. (Plate 3, fig. 4).

Prosternum with 4+4 teeth of which the second from the mesal incision on each side is the largest, the third being next.

Ventral plates without a trace of longitudinal sulci. Each with three distinct, pit-like, or more or less longitudinal, impressions arranged in a triangle with the apex cephalad, and with three short longitudinal impressions in a transverse row in front of the caudal margin. Last ventral plate strongly narrowed caudad. Truncate caudad with the corners a little rounded. Impressed with a distinct median longitudinal furrow. (Plate 3, fig. 3).

Coxopleurae of last legs without any true process, being a little roundly extended caudad; without any spines.

Length cir. 55 mm.

Locality.—State of Matto Grosso: Madeira-Mamore R. R. camp 41, on the Rio Madeira! (W. M. Mann).

One specimen was secured.

¹ On one side of type specimen there are but thirteen articles in the antenna, this being due, apparently, to breaking off of the antenna with subsequent regeneration of the distal article.

OTOSTIGMUS TIDIUS, sp. nov.

Brown, of more or less ferruginous tinge caudad and also being darker cephalad; plates mostly darker along caudal edges. Antennae very dark.

Head finely densely punctate. With no true sulci; but on the anterior portion an unusually deep median longitudinal furrow and also a similar one caudad of the middle with on each side of the latter a short, more shallow, furrow diverging from it cephalad.

Antennae composed of seventeen articles of which the first two are glabrous.

Prosternal teeth 4+4; the three innermost on each side nearly on a level and about equal in size, but the most ectal one situated more proximad, being at about the middle of lateral edge of dental plate. Process of first joint of prehensors notched or toothed on mesal side below apex. (Plate 2, fig. 6).

All dorsal plates with a distinct median longitudinal furrow, on each side of which, in most of the plates, there is a double longitudinal ridge between the two edges or keels of which lies the longitudinal sulcus of the corresponding side. Ectad of this double keel there is a much lower, often indistinct, keel. Plates longitudinally deeply fluted or furrowed along each lateral margin, producing the appearance of margination; but only the twenty-first plate truly margined. The keels are not well indicated on the first three or four plates. The median sulci are distinct from the fourth or fifth plates caudad. Last plate with caudal edge moderately bowed caudad. The median furrow distinct. An elevation or ridge each side of the middle divided by a weak furrow corresponding to that on the more anterior plates. (Plate 2, fig. 7).

Ventral plates with indications of the longitudinal sulci over the anterior portion, but the traces very short. Without any distinct pits or similar depressions. Last ventral plate strongly narrowed caudad. Caudally convexly rounded, not at all mesally incurved. A rather fine median longitudinal furrow present. (Plate 2, fig. 8).

Coxopleurae without true processes; but a little extended caudad, the corner being well rounded.

The first to the eighteenth pairs of legs with two tarsal spines, but the lateral spine on the eighteenth minute and that of the seventeenth intermediate in size. Nineteenth and twentieth legs with but a single tarsal spine.

Length cir. 14.5 mm.

Locality.— State of Amazonas: Manáos! (Mann and Baker).
One specimen.

OTOSTIGMUS REX, sp. nov.

Dorsum, excepting the first plate, dark olive, the plates somewhat paler along the caudal borders. Head and first dorsal plate conspicuously and abruptly different in color, being clear brown or somewhat testaceous, the head darker in middle region and in a narrow band running ecto-caudad on each side. Antennae and anal legs conspicuously rosaceous in color, the pairs of legs immediately preceding the last more weakly tinged with this color; other legs very pale clear brownish, weakly tinged with greenish. Prosternum clear brown. Venter similar to legs, darkest anteriorly.

Cephalic plate punctate the punctae very fine and rather weak.

Antennae composed of seventeen articles of which the first two and a half are glabrous and shining.

Prosternum with each dental plate bearing a distinctly separated tooth at each ectal end; but with the other teeth thoroughly fused into a continuous plate with no or but obscure traces of the separate ones. The longitudinal sulcus between the two plates of moderate depth.

Dorsal plates from the sixth on with very fine paired longitudinal sulci extending entire length of plate. From the third plate caudad there are longitudinal depressions mesad of each lateral margin which become deeper in caudal region and thus more sharply separating off the edge or simulating margination. From the third plate caudad a flat median keel is indicated, this on the anterior plates being obscure but posteriorly becoming more distinctly set off by the deepening of the limiting furrows on each side of it. Plates, especially the more caudal ones, rugose in the lateral depressions, the anterior ones otherwise smooth; but the posterior plates, and especially the last five or six, while appearing to the naked eye rather smooth, under the lens are seen to be finely scabrous, bearing over the entire surface, including edges and keel, rows of small, elevated, spinous points. Last dorsal plate bowed out caudad, the extended portion convexly rounded. In front of mesal portion of caudal edge a conspicuous and deep, pit-like, depression from the anterior edge of which a median keel runs cephalad across the plate; surface finely scabrous as on the other plates.

Ventral plates without longitudinal sulci. From the third or, more

indistinctly, the second to the twentieth plate with three pits, mostly deep and distinct, arranged in the form of a triangle with the median one cephalad, the three more or less clearly connected by more shallow depressions giving sometimes the appearance of a single V- or U-shaped impression. In addition there is a transverse row of three pit-like depressions in front of the caudal margin as in various related species, these pits on some of the more caudal plates lying in a more or less distinct transverse furrow. Last ventral plate long; conspicuously narrowed caudad; truncate or slightly indented at the middle. A distinct longitudinal median sulcus across the entire length.

Coxopleurae very slightly extended at mesocaudal corners, where they are wholly unarmed.

First legs with two tarsal spines. Second to nineteenth pairs of legs with a single tarsal spine; twentieth pair with no tarsal spine. Anal legs of moderate length. Prefemur clavately widening from base distad. Wholly smooth.

Length cir. 78 mm.

Locality.—State of Matto Grosso: Madeira-Mamore R. R. camp, 39, on the Rio Madeira! (Mann and Baker).

One specimen.

This species lies in the group of forms closely allied with *scabricaudus* in which the females are not easily distinguishable. The coloration of the present species is of a characteristic type similar to that of some Scolopendras and also present in *O. caudatus* and in several African species of this genus; in these forms the head and first dorsal plate being abruptly and conspicuously different in color from the rest of the dorsum. The species also differs from *scabricaudus* in being less strongly scabrous, in having no tarsal spine on the twentieth legs, in having a larger proportion of the third antennal article glabrous, and in having all the teeth excepting the most ectal on each side of the prosternum thoroughly fused together. The type is larger than the maximum measurement recorded for *scabricaudus* (70 mm.).

OTOSTIGMUS CASUS, sp. nov.

Olive-green in color above, brighter along the caudal margins of plates. Head more brown. Antennae brown of greenish caste, the first two articles clearer green. Legs pale brown of dilute greenish tinge. Venter lighter olive, the last ventral plate and the coxopleurae more brownish. Prosternum light greenish brown.

Head subdensely punctate, the punctae being moderately fine and not sharply impressed or limited.

Antennae composed of seventeen articles of which the first two are glabrous and shining, the others being densely pubescent as usual.

Dorsal plates from the fifth to the twentieth with the two longitudinal sulci present and complete; fine. Lateral portions of plate from the fifth caudad depressed leaving the lateral margin distinctly elevated, especially in the middle and caudal regions, but true margination present only on the twenty-first plate. The depressed lateral portion of the plate rugose, the main rugae being longitudinal. The elevated margins, the rugae, and, less extensively, the intermediate surface, roughened with series of numerous spinulose points. From the fifth or sixth plates on a median longitudinal keel is indicated, this being at first obscure but becoming more and more distinct caudad, while at the same time on each side of it and just mesad of the sulcus appears another keel, the three keels being distinct on the caudal segments; the keels are scabrous like the lateral portions of the plates. Last dorsal plate with the posterior edge moderately bowed out caudad and mesally truncate. With three longitudinal keels corresponding to those of the other plates extending from the anterior margin caudad two thirds the length of the plate, the plate caudad of their ends having a shallow pit-like depression. Keels and general surface scabrous.

Sulci of ventral plates detectable only as very short traces at the anterior border of each. With three pit-like depressions arranged in a triangle as usual, these being of but moderate depth and size and not coalesced. In addition there are three other depressions along the caudal border separated from those of the triangle by a distinct transverse sulcus. On some of the more caudal plates the anterior median pit may be extended a considerable distance caudad as a median furrow. Last ventral plate conspicuously narrowed caudad, the sides being convex at anterior ends but straight for most of their length. Caudal margin with lateral halves straight and meeting in the middle in a slightly reentrant angle. No distinct median sulcus present.

Coxopleurae a little extended caudad at caudomesal corners which are simply rounded, no distinct process being developed, wholly unarmed.

Only the first pair of legs with two tarsal spines. Second to eighteenth pairs with a single tarsal spine. Nineteenth to twenty-first pairs unknown, being absent from the only specimen known.

Length 57 mm.

Locality.— State of Matto Grosso: Madeira-Mamore R. R. camp 39, on the Rio Madeira! (W. M. Mann).
One specimen.

OTOSTIGMUS GOELDI Brölemann.

Ann. Soc. ent. France, 1898, **67**, p. 249, pl. 20, fig. 2; Kraepelin Revis. Scolop., 1903, p. 128; Brölemann, Cat. Myr. Brésil, 1909, p. 12.

Locality.— State of Para: Para (sec. Brölemann).

OTOSTIGMUS SCABRICAUDUS (Humbert and Saussure).

Branchiosloma scabricauda Humbert et Saussure, Rev. mag. zool., 1870, p. 203; Saussure et Humbert, Études Myr., 1872, p. 121, pl. 2, fig. 15, etc.; Kohlrausch, Archiv. naturg., 1881, **47**, 1, p. 75.

Otostigma appendiculatus Porat, Bih. Svensk. vet. akad. Handl., 1876, **4**, p. 23.

Otostigma brasiliense Meinert, Vid. Medd. nat. foren, Kjøbenhavn, 1884, p. 119.

Otostigma brasiliensis Karsch, Berl. ent. zeitschr., 1888, p. 31.

Otostigma scabricaudus Pocock, Ann. mag. nat. hist., 1890, ser. 6, **6**, p. 142, Brölemann, Mem. Soc. zool. France, 1900, **13**, p. 96; Rev. Museu Paulista, 1901, **5**, p. 40; Kraepelin, Revis. Scolop., 1903, p. 126, fig. 61; Brölemann, Cat. Myr. Brésil, 1909, p. 13.

Localities.— State of Bahia: Bahia; State of Minas Geraes; State of Rio de Janeiro: Rio de Janeiro (type locality), Rio Espirito Santo; State of São Paulo: Alto da Serra, Piquete.

OTOSTIGMUS CAUDATUS Brölemann.

Rev. Mus. Paulista, 1901, **5**, p. 39, pl. 1, fig. 1-3; Kraepelin Revis. Scolop., 1903, p. 132, fig. 71, 72; Brölemann, Cat. Myr. Brésil, 1909, p. 12.

Localities.— State of São Paulo: São Paulo, Belém, Alto da Serra, Itapetininga.

OTOSTIGMUS TIBIALIS Brölemann.

Rev. Mus. Paulista, 1901, 5, p. 39, fig. 4, 5.

Otostigmus caudatus tibialis Kraepelin, Revis. Scolop., 1903, p. 132, fig. 73, 74.

Otostigmus caudatus Brölemann, Cat. Myr. Brésil, 1909, p. 13.

Localities.—State of São Paulo: São Paulo, Piquete, Alto da Sérra, Santos; State of Parana.

TREMATOPHYCUS Peters.

Reise Mozambique, 1862, 5, p. 519.

Branchiostoma Newport (*nom. preocc.*) Trans. Linn. soc. London, 1844, 19, p. 411; Meinert, Proc. Amer. philos. soc., 1886, 23, p. 182.

Ptychotrema Peters (*nom. preocc.*), Monatsb. Berl. akad., 1855, p. 82.

Rhysida Wood, Journ. Acad. nat. sci. Phil., 1862, ser. 2, 5, p. 40; Pocock, Ann. mag. nat. hist., 1891, ser. 6, 7, p. 58; Kraepelin Revis. Scolop., 1903, p. 139; Verhoeff, Bronn's Thierreich, 1907, 5, p. 57.

Ethmophora Pocock, Ann. mag. nat. hist., 1891, ser. 6, 7, p. 58.

Of this genus three species are at present known from Brazil, and a fourth is practically certain to occur there and is accordingly introduced into the following key.

Key to Species.

- a. At least some of the dorsal plates with the paired submedian sulci crossing entire length of plate.
 - b. Only the twenty-first dorsal plate margined; prefemur of anal leg with one (or two) ventral spines.
 - T. nudus* (Newport).
 - bb. Some of the dorsal plates cephalad of the twenty-first also distinctly margined.
 - c. Prefemur of the anal legs wholly unarmed; process of coxopleurae short, subtriangular, without lateral spines.
 - T. celeris* (Humbert and Saussure).
 - cc. Prefemur of anal legs armed with eight to thirteen spines; process of anal coxopleurae long, with one or two lateral spines in addition to the terminal ones.
 - T. longipes* (Newport).
 - aa. None of the dorsal plates with sulci crossing its entire length, these appearing at most as short traces at ends of plates.
 - Prefemur of anal legs wholly unspined.
 - T. brasiliensis* (Kraepelin).

TREMATOPHYCUS NUDUS (Newport).

Branchiostoma nudum Newport, Trans. Linn. soc. London, 1844, **19**, p. 412.

Branchiostoma gymnopus Kohlrausch, Arch. naturg., 1881, **47**, p. 67.

Branchiostoma gymnopus ceylonicum Haase, Abhandl. Mus. Dresden, 1887, **5**, p. 86.

Rhysida immarginata var. Pocock, Biol. Centr. Amer. Chilopoda, 1896, p. 26.

Rhysida nuda Kraepelin, Revis. Scolop., 1903, p. 144.

Locality.—While not specifically recorded from Brazil, it is distributed widely in Mexico and Central America as well as in Paraguay, so that its occurrence in Brazil is practically certain.

TREMATOPHYCUS LONGIPES (Newport).

Branchiostoma longipes Newport, Trans. Linn. soc. London, 1844, **19**, p. 411; Haase, Abhandl. Mus. Dresden, 1887, **5**, p. 84.

Branchiostoma obsoletum Porat. Bih. Svensk. vet. akad. Handl., 1876, **4**, no. 7, p. 25.

Branchiostoma gracile Kohlrausch, Archiv naturg., 1881, **47**, **1**, p. 66.

Branchiostoma affine Kohlrausch, Ibid., p. 67.

Branchiostoma longipes rotundatum Haase, loc. cit.

Rhysida longipes Pocock, Biol. Centr. Amer. Chilopoda, 1896, p. 27; Kraepelin, Revis. Scolop., 1903, p. 148, fig. 91; Brölemann, Cat. Myr. Brésil, 1909, p. 14.

Localities.—"Brazil" (M. C. Z. coll.; also W. M. Mann, without precise locality); State of Bahia.

TREMATOPHYCUS CELERIS (Humbert and Saussure).

Branchiostoma celer Humbert et Saussure, Rev. mag. zool., 1876, ser. 2, **22**, p. 202; Kohlrausch, Arch. naturg., 1881, **47**, **1**, p. 69, Meinert, Proc. Amer. philos. soc., 1886, **23**, p. 183.

Rhysida celeris Silvestri, Mus. zool. anat. comp. R. univ. Torino, 1895, **10**, p. 23; Pocock, Biol. Centr. Amer. Chilopoda, 1896, p. 27; Kraepelin, Revis. Scolop., 1903, p. 149; Brölemann, Cat. Myr. Brésil, 1909, p. 14.

Localities.—State of Matto Grosso: Madeira-Mamore R. R. camp 39, on the Rio Madeira!, Abuná, Bolivia! (W. M. Mann); State of Rio Grande do Norte: Ceará-Mirim! (Mann and Heath. The specimen from this locality variant).

TREMATOPHYCUS BRASILIENSIS (Kraepelin).

Rhysida brasiliensis Kraepelin, Revis. Scolop., 1903, p. 152, fig. 95, 96.

Localities.— State of Minas Geraes; State of Rio de Janeiro: Rio Espirito Santo.

SCOLOPENDRIDAE.

Of this family the genera Scolopendropsis, Cupipes, Cormocephalus (probably introduced), Hemiscolopendra, and Scolopendra are known in the Brazilian fauna.

SCOLOPENDROPSIS Brandt.

Bull. sci. Acad. imper. sci. St. Peterburg, 1840, 7, p. 24; Kraepelin Revis. Scolop., 1903, p. 179; Verhoeff, Bronn's Thierreich, 1907, 5, p. 50.
Rhoda Meinert, Proc. Amer. philos. soc., 1886, 23, p. 188.
Pithopus Pocock, Ann. mag. nat. hist., 1891, ser. 6, 7, p. 223; Kraepelin, Revis. Scolop., 1903, p. 171; Verhoeff, Bronn's Thierreich, 1907, 5, p. 259.

Two species, the only valid ones known, occur in Brazil.

SCOLOPENDROPSIS BAHIENSIS Brandt.

Bull. sci. Acad. imper. sci. St. Peterburg, 1840, 7, p. 24; Kraepelin, Revis. Scolop., 1903, p. 171; Brölemann, Cat. Myr. Brésil, 1909, p. 31.
Rhoda thayeri Meinert, Proc. Amer. philos. soc., 1886, 23, p. 188.
Pithopus inermis Pocock, Ann. mag. nat. hist., 1891, ser. 6, 7, p. 223, pl. 5, f. 5; Kraepelin, Revis. Scolop., 1903, p. 172.
Scolopendropsis thayeri Brölemann, Cat. Myr. Brésil, 1909, p. 32.

Localities.— State of Bahia: Bahia, Iguarassu (the type locality of *inermis* (Pocock)). State of Para: Santarem (the type locality of *thayeri* (Mein.)).

Kraepelin suggests the identity of *thayeri* Meinert with *calcaratus* Pocock; but an examination of the type of *thayeri* shows that it is the same as the *inermis* of Pocock and that both are the same as Brandt's *bahiensis* which has long priority. Through probable error twenty-three pairs of legs were attributed to Brandt's species, although the not very probable suggestion has been made that the species is dimorphic, having some individuals with twenty-three and others with twenty-one pairs of legs.

SCOLOPENDROPSIS CALCARATUS (Pocock).

Pithopus calcaratus Pocock, Ann. mag. nat. hist., 1891, ser. 6, 7, p. 224, pl. 5, fig. 3.

Scolopendropsis calcaratus Brölemann, Ann. Soc. ent. France, 1902, 71, p. 651; Cat. Myr. Brésil, 1909, p. 32.

Localities.— State of Bahia: Bahia (type locality), Santo Antonio da Barra; State of Rio Grande do Norte: Natal! (W. M. Mann). Ceará-Mirim! (Mann and Heath).

CUPIPES Kohlrausch.

Archiv. naturg. 1881, 47, 1, p. 78; Kraepelin, Revis. Scolop., 1903, p. 174; Verhoeff, Bronn's Thierreich, 1907, 8, p. 260.

Key to Species.

- a. Femur or second joint of anal legs armed dorsally at distal end with two bifid spines, one on each side of the longitudinal furrow.
C. brasiliensis Humbert and Saussure.
- aa. Femur of anal legs unarmed.
 - b. Only the twenty-first dorsal plate distinctly margined.
 - c. Most legs with a tarsal spine; last ventral plate parallel sided, not narrowed caudad. *C. spinifer* Kraepelin.
 - cc. All legs without tarsal spines; last ventral plate narrowed caudad.
 - d. Prefemur of anal legs with about five to eleven spines distributed over its surface. . . . *C. unguatis* Meinert.
 - dd. Prefemur with only two spines, these being those at dorsomesal angle of distal end.
C. unguatis mitis Brölemann.
 - bb. Plates from seventh to tenth caudad also margined as well as the twenty-first.
 - c. Coxopleura with a short but distinct process at meso-caudal corner (Plate 2, fig. 9); each dental plate of prosternum with an elongated ectal process and a single mesal tooth at base of this (Plate 3, fig. 5); head distinctly longer than wide. *C. amazonae*, sp. nov.
 - cc. Coxopleura with no such process, simply rounded at meso-caudal angle (Plate 3, fig. 8); each dental plate with four subsimilar teeth of the usual form (Plate 3, fig. 7); head equal in length and breadth. *C. neglectus*, sp. nov.

CUPIPES BRASILIENSIS (Humbert and Saussure).

Cormocephalus brasiliensis Humbert et Saussure, Rev. mag. zool., 1870, ser. 2, 22, p. 203; Saussure et Humbert, Etude Myr. 1872, p. 124, pl. 6, fig. 17.
Cupipes brasiliensis Brölemann, Cat. Myr. Brésil, 1909, p. 8.

Locality.— Brazil.

This species was regarded, doubtfully, as the same as *C. unguлатis* Meinert by Meinert as well as by Kraepelin. But, as pointed out by Brölemann, and as indicated in the key above, the presence of the dorsal spines on the second joint of the anal legs seems clearly to separate *brasiliensis* from Meinert's species. At any rate, until the Brazilian fauna is better known, or specimens of *unguлатis* are shown to present the important variation mentioned, it would be quite premature to ignore this characteristic.

CUPIPES SPINIFER Kraepelin.

Revis. Scolop., 1903, p. 177, fig. 117.

Locality.— State of Pará: Pará (type locality).

Known from a single specimen in the Hamburg Museum.

CUPIPES UNGULATIS Meinert.

Proc. Amer. Philos. soc., 1886, 23, p. 187; Brölemann, Rev. Museu Paulista, 1903, 6, p. 64; Cat. Myr. Brésil, 1909, p. 9.

Localities.— State of Pernambuco: Pernambuco! (type locality); State of Amazonas: Manáos (sec. Brölemann).

CUPIPES UNGULATIS MITIS Brölemann.

Rev. Museu Paulista, 1903, 6, p. 65; Cat. Myr. Brésil, 1909, p. 9.

Locality.— State of Amazonas: Manáos (type locality).

This and the preceding form are known at present from too few specimens to be able properly to judge of their precise relationship. Considerable variation has already been noted in the spining of the prefemur of *C. unguлатis*; and it may prove not possible to segregate the forms on the basis of this character.

CUIPIPES AMAZONAE, sp. nov.

Dorsum olive or olive-brown; most of the plates of the middle and anterior regions of body with a blackish spot or short stripe at each lateral margin; in the plates of the median region also a dark spot on caudal border at caudal end of each sulcus or the two spots may be united as a transverse band. Head darker, more brownish and dusky. Antennae bright green. Prosternum and prehensorial feet clear brown, the latter rufous laterally and especially distally proximad of the black claw proper.

Head clearly longer than wide (46:41). Sides a little convex just behind the eyes and then substraight and a little converging to the caudal corners. Finely and uniformly subdensely punctate. The two diverging longitudinal sulci reaching or very nearly reaching the anterior margin, each terminating in a transverse sulcus slightly removed from the edge of antennal socket with which it runs parallel.

Antennae composed of seventeen articles of which the first seven are glabrous or practically so and in this respect sharply separated from the more distal group.

Prosternum with two sharply defined longitudinal sulci converging cephalad and uniting at an angle at anterior end; these sulci crossed by a transverse sulcus which is branched and has anastomoses as shown in Plate 3, fig. 5. Dental plate without true teeth, but bearing an elongate ectal process with on mesal side at base a low, dark, denticiform elevation (Plate 3, fig. 5); on one side the plate is malformed as shown in the figure, this probably being due to injury with imperfect subsequent regeneration.

Margination of the dorsal plates indicated from about the seventh caudad but not very distinctly until the tenth. Plates with an obscure low median keel defined by two indistinct longitudinal furrows; also a vague furrow laterad of each median sulcus may be indicated. Last dorsal plate with a sharply impressed median longitudinal sulcus.

Second to twentieth ventral plates with the usual two longitudinal sulci crossing the plate. A rather vague transverse furrow may be traced at the level of the legs; while on some plates indications of a very weak median longitudinal furrow may be detected. Last ventral plate clearly wider at its anterior end than long (2.6:2). Sides strongly converging caudad; plate truncate caudad, the corners a little rounded. Without a median sulcus or furrow. (Plate 2, fig. 9).

Coxopleurae with a short but distinct process at mesocaudal corner,

the process bearing two spines; not spined on coxopleural margin laterad of the process proper. Porose area not fully reaching the caudal margin. (Plate 2, fig. 9).

Anal legs with articles proximad of the tarsus much thickened, especially from side to side as usual. Prefemur and especially the femur deeply longitudinally furrowed dorsally at distal ends. Prefemur with a distinct spine at mesodistal angle on dorsal side; two small spines near upper edge of mesal surface and three more ventral, two being at the distal end ventrad of the corner spine and one toward the proximal end; in addition there are four strictly ventral spines, two in each of two rows. Femur wholly unarmed.

Length cir. 43 mm.

Locality.—State of Amazonas: Manáos! (Mann and Baker).

One specimen.

CUPIPES NEGLECTUS, sp. nov.

Dorsum brown. Head olivaceous. Antennae bluish green. Legs pale.

Head equal in length and breadth. Sulci distinct, diverging cephalad and each reaching the margin at the eye. Punctae scattered.

Antennae composed of seventeen articles. In the type the antennae are considerably rubbed; but apparently the first article is wholly glabrous and the second one nearly so, the third and fourth with an intermediate number of hairs and the fifth and subsequent ones with the full complement.

Prosternum with two longitudinal submedian sulci which meet at an angle anteriorly; not crossed by any distinct transverse lines. Dental plates bearing 4+4 teeth which are of normal form and long and acute; the two intermediate teeth on each side longest; the most ectal tooth situated distinctly more proximad (Plate 3, fig. 7).

Dorsal plates margined from the eighth or ninth segment caudad, the margination becoming more and more distinct in going toward the caudal end. Sulci continuous and very distinct on all the plates excepting the last. First to third plates with a median longitudinal furrow; the fourth plate; those from the fifth caudad with a low median longitudinal keel which is flat and set off by two shallow furrows in the usual way. Last dorsal plate with a sharply impressed median longitudinal sulcus.

Ventral plates from the second to the twentieth distinctly bisulcate. Some of the plates showing an indistinct median depression on caudal

portion. Last ventral plate narrowed caudad; sides straight; caudal margin also straight. A little wider across anterior end than long (ratio cir. 39:37). With a rather weak median longitudinal sulcus.

Coxopleurae of last legs not at all produced caudad. Bearing one or two closely approximate spines at mesocaudal angle; none ectad of this. Porose area not reaching caudal margin by a considerable distance.

Length of the two types 26 and 55 mm. respectively.

Joints of the anal legs proximad of the tarsus strongly crassate as usual. The first three joints longitudinally dorsally sulcate, the sulci most distinct at distal ends and on the first two joints. Spines of prefemur very small and difficult to detect; two present close together on dorsomesal corner of distal end; a single one in line with the preceding two farther cephalad and three on the mesal surface were detected. First twenty pairs of legs without tarsal spines.

Locality.—State of Matto Grosso: Madeira-Mamore R. R. camp 39, on the Rio Madeira! (W. M. Mann).

Two specimens.

Evidently this species is related to *C. impressus* (Porat); but it may be distinguished in having the cephalic plate equal in length and breadth instead of distinctly longer (ratio 4:3), by having the last ventral plate with a median longitudinal furrow, by having fewer articles of the antennae glabrous and these less abruptly differentiated from the others, etc.

CORMOCEPHALUS Newport.

Trans. Linn. soc. London, 1844, 19, p. 275, 419; Meinert, Proc. Amer. philos. soc., 1886, 23, p. 205; Kraepelin, Revis. Scolop., 1903, p. 184; Verhoeff, Bronn's Thierreich, 1907, 5, p. 262.

Rhomboccephalus Newport (in part) Trans. Linn. soc. London, 1844, 19, p. 275, 425.

This is a typically Australian, Indian, and African genus, the single species recorded from America having probably been introduced from ships.

CORMOCEPHALUS AURANTIIPES (Newport).

Scolopendra aurantiipes Newport, Ann. mag. nat. hist., 1844, 13, p. 99.

Scolopendra subminiata Newport, Ibid., p. 100.

Cormocephalus aurantiipes Newport, Trans. Linn. soc. London, 1844, 19, p. 420;

Haase, Abhandl. Mus. Dresden, 1887, 5, p. 57; Kraepelin, Revis. Scolop., 1903, p. 197; Brölemann, Cat. Myr. Brésil, 1909, p. 7.

Cormocephalus obscurus Newport, Loc. cit., p. 420.

Cormocephalus subminiatus Newport, Loc. cit., p. 420.

Cormocephalus miniatus Newport, Loc. cit., p. 420.

Rhombcephalus brevis Newport, Loc. cit., p. 426.

Scolopendra obscura Gervais, Insect. Aptères, 1847, 4, p. 272.

Scolopendra miniata Gervais, Ibid.

Scolopendra subminiata Gervais, Ibid.

Scolopendra brevis Gervais, Ibid.

Cormocephalus gracilis Kohlrausch, Archiv. naturg., 1881, 47, 1, p. 86.

Cormocephalus pygomelas Kohlrausch, Ibid., p. 90.

Cormocephalus aurantiipes spinosus Haase, Loc. cit., p. 58.

Localities.—State of Rio de Janeiro: Rio de Janeiro! M. C. Z.; Brazil (without more definite locality, recorded as *C. gracilis* and *C. pygomelas* by Kohlrausch.) Reported also from Guatemala by Pocock.

HEMISCOLOPENDRA Kraepelin.

Revis. Scolop., 1903, p. 212; Verhoeff, Bronn's Thierreich, 1907, 5, p. 261.

HEMISCOLOPENDRA LAEVIGATA (Porat).

Cormocephalus laevigatus Porat, Bih. Svensk. vet. akad. Handl., 1876, 4, no. 7, p. 17.

Scolopendra cormocephalina Kohlrausch, Archiv. naturg., 1881, 47, 1, p. 123.

Scolopendra longispina Meinert, Proc. Amer. philos. soc., 1886, 23, p. 199; Brölemann, Cat. Myr. Brésil, 1909, p. 19.

Scolopendra appendiculata Daday, Termes, füzetek., 1891, 14, p. 152.

Localities.—State of São Paulo: Belém.

SCOLOPENDRA Linné.

Syst. nat. ed. 10, 1758, 4, p. 637; Newport, Trans. Linn. soc. London, 1844, 19, p. 275, 377; Latzel, Myr. Ost.-Ung. monarch., 1880, 1, p. 137; Meinert, Proc. Amer. philos. soc., 1886, 23, p. 190; Vid. Meddl. nat. foren. Kjöbenhavn, 1886, p. 125; Kraepelin, Revis. Scolop., 1903, p. 223; Verhoeff, Bronn's Thierreich., 1907, 5, p. 263.

Eight valid species, one of them new, are recognized in this paper as occurring in Brazil. Brölemann lists *S. viridis* Wood as occurring

in Brazil, fide Meinert; but a reference to the paper cited fails to reveal any record of the species from the country by the latter author and the species is accordingly here omitted. The names falling as synonyms in this genus are very numerous. The known Brazilian species may be separated by means of the following key.

Key to Species.

- a. First dorsal plate with a deeply impressed transverse cervical sulcus which may be nearly covered by the head.
- b. Prefemur of the twentieth legs (as also sometimes of some of the immediately preceding pairs) armed on dorsal side at distal end one to several spines; prosternum with a fine sulcus across anterior portion.
- c. Dorsal plates from the sixth or seventh caudad distinctly margined laterally.
 - d. At least eight or ten basal articles of the antennae glabrous, the others finely pubescent, the hairs not in rows; coxopleural process with nine spines or points. *S. gigantea* Linné.
 - dd. Only four or five basal articles of antennae glabrous; hairs of the others often in streaks or rows; points of the coxopleural process fewer.
 - e. Ventral plates without longitudinal sulci; margination of dorsal plates beginning at seventh segment; femur of twentieth and also of nineteenth legs with one or two spines at distal end above.
 - S. angulata* Newport.
 - ee. Ventral plates of second to twentieth segments with two deep longitudinal sulci crossing the entire length of plate; margination of dorsal plates beginning with the fifth; femur of twentieth legs lacking spine at distal end above.
 - S. viridicornis* Newport.
- cc. Margination of dorsal plates beginning only with the eleventh to fourteenth segment.
 - Four basal articles of antennae glabrous, the others densely pubescent; none of dorsal plates with sulci passing across entire length; claw of anal legs with two minute basal spines. *S. explorans*, sp. nov.

- bb. Prefemur of twentieth legs not at all armed at distal end above; prosternum with no transverse sulcus across entire width of anterior portion.

Head wholly without longitudinal sulci; last dorsal plate with a median longitudinal sulcus; antennae composed of from twenty-five to thirty-one articles of which the eighth to seventeenth proximal ones are glabrous.

S. polymorpha Wood.

- aa. First dorsal plate without any such deeply impressed transverse cervical sulcus.

- b. Prefemur of nineteenth and twentieth pairs of legs armed dorsally at distal end with from one to six spinous teeth; head with longitudinal sulci.....*S. alternans* Leach.

- bb. Prefemur of nineteenth and twentieth pairs of legs unarmed at distal end above; head without longitudinal sulci.

- c. Prefemur of anal legs armed ventrally with from six to nine spines; last dorsal plate with a fine median longitudinal sulcus.....*S. morsitans* Linné.

- cc. Prefemur of anal legs with only three, or less, ventral spines or sometimes (in varieties) with none; last dorsal plate without a median sulcus.....*S. subspinipes* Leach.

SCOLOPENDRA GIGANTEA Linné.

Syst. nat. ed. 10, 1758, 1, p. 638; Kraepelin, *Revis. Scolop.*, 1903, p. 233; Brölemann, *Cat. Myr. Brésil*, 1909, p. 17.

Scolopendra gigas Leach, *Trans. Linn. soc. London*, 1814, 11, p. 383; Meinert, *Proc. Amer. philos. soc.*, 1886, 23, p. 191.

Scolopendra insignis Gervais et Goudot, *Ann. Soc. ent. France*, 1844, 2, p. 29.

Scolopendra prasinipes Wood, *Proc. Acad. nat. sci. Phil.*, 1862, p. 11.

Scolopendra epileptica Wood, *Ibid.*

Scolopendra annulipes Lucas, *Bull. Soc. ent. France*, 1884, ser. 6, 4, p. 74; Brölemann, *Cat. Myr. Brésil*, 1909, p. 18.

Localities.—State of Amazonas: Obidos! James and Hunnewell, Nathaniel Thayer expedition. M. C. Z.; State of Para: Santarem! (Chas. Linden, M. C. Z.); State of Pernambuco: Villa Bella! (J. C. Fletcher; M. C. Z.). Lucas's type of *S. annulipes* was also from Brazil, the definite locality not being indicated.

SCOLOPENDRA ANGULATA Newport.

- Ann. mag. nat. hist., 1844, 13, p. 97; Pocock, Journ. Linn. soc. London, 1893, 24, p. 146; Brölemann, Cat. Myr. Brésil, 1909, p. 17.
Scolopendra puncticeps Wood, Proc. Acad. nat. sci. Phil., 1862, p. 14.
Scolopendra punctiscuta Wood, Ibid.
Scolopendra prasina C. L. Koch, Myr. 1864, 2, p. 23, fig. 146; Meinert, Proc. Amer. philos. soc., 1886, 23, p. 192.
Scolopendra nitida Porat, Bih. Svensk. vet. akad. Handl., 1876, 4, no. 7, p. 8.
Scolopendra respublicana Giebel, Zeitschr. ges. naturw., 1879, 52, p. 326.

Localities.—State of Matto Grosso: Madeira-Mamore, R. R. camp 41, on the Rio Madeira! (W. M. Mann); Brazil (without more definite locality, reported by Porat as *nitida* by Pocock and by Kraepelin).

The species is widely distributed elsewhere in South America and in the Antilles.

SCOLOPENDRA VIRIDICORNIS Newport.

- Ann. mag. nat. hist., 1844, 13, p. 97; Kraepelin, Revis. Scolop., 1903, p. 236; Brölemann, Cat. Myr. Brésil, 1909, p. 9.
Scolopendra punctidens Newport, Loc. cit.
Scolopendra cristata Newport, Loc. cit.; Meinert, Proc. Amer. philos. soc., 1886, 23, p. 192.
Scolopendra variegata Newport, Loc. cit., p. 98.
Scolopendra hopei Gray, List Myr. Brit. mus., 1844, p. 45.
Scolopendra herculeana C. L. Koch, Myr., 1863, 1, p. 22, fig. 20.
Scolopendra morsitans C. L. Koch, Ibid., p. 37, fig. 33.
?Scolopendra costata C. L. Koch, Myr., 1863, 2, p. 25, fig. 147.

Localities.—State of Rio Grande do Norte: Natal! (W. M. Mann); State of Pará: Pará; State of Bahia: Bahia, Rio São Francisco (sec. Koch); State of Pernambuco; State of Amazonas: Manáos, Amazon River! (J. C. Fletcher, M. C. Z.); State of Ceará: Ceará or Fortaleza! (W. M. Mann); Brazil without definite locality (Chas. Linden, M. C. Z.; and sec. Porat and Kohlrausch, as *S. cristata*; Gray, as *S. hopei*; and Newport and Kohlrausch, as *viridicornis*).

Judging by the number of specimens secured by Mr. Mann, the species is very common at Natal.

SCOLOPENDRA EXPLORANS, sp. nov.

Dorsum dark olive, the plates being somewhat darker at the caudal borders and in a transverse row of areas or spots across the anterior half. Head darker excepting along the caudal border where it is much lighter, somewhat testaceous. Antennae olivaceous. Legs testaceous, excepting the posterior pairs, especially the ultimate, which are of a somewhat cherry-red color, particularly distad. Venter light brown. Prosternum dark brown; the prehensors somewhat rufous proximad of the black claws.

Cephalic plate with two very fine submedian sulci diverging cephalad across entire length of plate. Very finely and subdensely uniformly punctate.

Antennae of moderate length. Composed of seventeen articles of which the first four are subglabrous and subdensely finely punctate; the other articles very densely clothed with fine brownish pubescence. Articles from the sixth distad not more than one and a half times longer than wide.

Prosternum and prehensors subdensely punctate with fine punctae. With no median sulcus but with a distinct though fine transverse sulcus, caudad of which there is a mesal, shallow depression. Each dental plate with a large isolated tooth at ectal end and apparently three thoroughly fused teeth mesad of this, there being only slight indications of any divisions in the fused piece.

First dorsal plate with a distinct cervical sulcus. Very finely punctate, the punctae being rather more scattered than on the head. No sulci detected on the second plate. The third to twentieth plates with paired longitudinal sulci extending full length of plate, fine. Finely punctate like the first plate, but the punctae becoming fainter and fainter caudad. First indications of lateral margination shown on the eleventh to fourteenth plate, the margination becoming more and more strongly marked caudad. Last dorsal plate without a median keel. Not punctate. Convexly arched on anterior portion, the posterior being more flattened. Caudal margin rather strongly bowed out caudad, the border being depressed in front of the median portion of the margin.

Ventral plates either wholly without sulci or with very short traces of these at anterior border. Punctae fine, becoming faint caudad. Last ventral plate narrowed caudad. Caudal margin subtruncate, being but weakly rounded; mesally slightly notched or incurved. Without median longitudinal sulcus.

Caudal process of coxopleuræ of anal legs very short; ending in three or four spines or points, the coxopleuræ being otherwise unarmed. Pores very fine and numerous.

First pair of legs with two tarsal spines. Second to nineteenth pairs with a single tarsal spine. Prefemur of nineteenth legs dorsally at distal end with a single spine; that of the twentieth with two spines; the femur of the latter pair dorsally at distal end also with a single spine. Prefemur of twentieth legs unarmed ventrally.

Prefemur of anal legs with the corner process at distal end above ending in two stout points or teeth; ventrally with five spines arranged in two transverse or somewhat oblique rows, a distal row, composed of three spines, being at about the middle of length of joint, and a more proximal one of two spines (or in three longitudinal rows, 2, 2, 1); mesally with three or four spines in two longitudinal rows; and along dorsomesal edge with two spines in addition to a single one more strictly dorsal. Femur with two spines on proximal half along dorsomesal edge with a third one ventrad of these on the mesal surface; and, in addition, also a spine at distal end on mesodorsal corner. Claw with two basal spines which are very small.

Length cir. 83 mm.

Locality.—State of Matto Grosso: Madeira-Mamore R. R. camp 39, on the Rio Madeira! (W. M. Mann).

This interesting species is evidently close to *S. armata* described by Kraepelin from Venezuela (Maracaibo), with which it is characteristically separated from all others now known. Among the more important differences between these two species, so far as the description of *armata* permits of comparison, may be mentioned the complete absence of any spines on the twentieth legs; the dense pubescence of antennæ on all articles distad of the fourth; the distinct punctation of the first dorsal plate; the margination of the dorsal plates from the eleventh to fourteenth caudad instead of from the eighteenth or nineteenth; the absence of paired sulci passing entirely across any of the plates; the presence of two spines at base of claw of anal legs instead of but one, etc.

SCOLOPENDRA POLYMORPHA Wood.

Proc. Acad. nat. sci. Phil., 1862, p. 11; Kraepelin, Revis. Scolop., 1893, p. 241.

Scolopendra copeiana Wood, Journ. Acad. nat. sci. Phil., 1862, ser. 2, 5, p. 27;

Pocock, Biol. Centr. Amer. Chilopoda, 1895, p. 19.

Scolopendra mysteca Humbert et Saussure, Rev. mag. zool., 1869, ser. 2, 21, p. 157.

Scolopendra pachypus Kohlrausch, Archiv. naturg., 1881, 47, 1, p. 113.

Scolopendra leptodera Kohlrausch, Ibid.; Brölemann, Cat. Myr. Brésil, 1909, p. 19.

Locality.—Brazil (type of *leptodera* Kohlrausch, which has been restudied by Kraepelin and identified with *polymorpha*).

This is a very common species in the southern United States and in Mexico. It has also been recorded from Venezuela.

SCOLOPENDRA ALTERNANS Leach.

Trans. Linn. soc. London, 1812, 11, p. 383; Meinert, Proc. Amer. philos. soc. 1886, 23, p. 193; Kraepelin, Revis. Scolop., 1903, p. 244; Brölemann, Cat. Myr. Brésil, 1909, p. 15.

Scolopendra morsitans Palisot de Beauvois (non Linné), Ins. Afr. Amer., 1805-1821, p. 152.

Scolopendra sagraea Gervais, Ann. sci. nat., 1837, ser. 2, 7, p. 50; Brandt, Bull. sci. Acad. imper. sci. St. Petersburg, 1840, 7, p. 57.

Scolopendra complanata Newport, Ann. mag. nat. hist., 1844, 13, p. 98.

Scolopendra grayii Newport, Ibid.

Scolopendra multispinata Newport, Ibid.

Scolopendra incerta, Newport, Trans. Linn. soc. London, 1844, 19, p. 404.

Scolopendra crudelis C. L. Koch, Syst. Myr., 1847, p. 387; Myr., 1864, 2, p. 36, fig. 158, 159; Meinert, Proc. Amer. philos. soc., 1886, 23, p. 194.

Scolopendra cubensis Saussure, Mém. Soc., phys. hist. nat. Genève, 1860, 15, p. 387.

Scolopendra testacea Wood, Journ. Acad. nat. sci. Phil., 1862, ser. 2, 5, p. 26.

Scolopendra torquata Wood, Ibid.

Scolopendra longipes Wood, Ibid.

Scolopendra alternans Meinert, Proc. Amer. philos. soc., 1886, 23, p. 193; Kraepelin, Revis. Scolop., 1903, p. 244; Brölemann, Cat. Myr. Brésil, 1909, p. 15.

Locality.—Brazil! (M. C. Z.).

This is a very common species in the West Indies, etc.

SCOLOPENDRA MORSITANS Linné.

Syst. nat. ed. 10, 1758, 1, p. 638; Kraepelin, Revis. Scolop. 1903, p. 250; Brölemann, Cat. Myr. Brésil, 1909, p. 19.

(For synonymy and bibliography cf. Kraepelin, Loc. cit.).

Localities.—State of Amazonas: Manáos; State of Parahyba: Parahyba! (Nathaniel Thayer expedition, M. C. Z.); State of

Pará: Pará! (Nathaniel Thayer expedition, M. C. Z.); State of Bahia: Santarem! (M. C. Z.); State of Rio de Janeiro: Rio de Janeiro! (M. C. Z.); Brazil, without definite locality; (recorded by Gervais as *brandtiana* and *platypoides*; by Saussure as *brandtiana*; by Porat as *longicornis*; also specimens in M. C. Z., with no more definite label).

This is a cosmopolitan species.

SCOLOPENDRA SUBSPINIPES Leach.

Trans. Linn. soc. London, 1814, 11, p. 383; Kraepelin, Revis. Scolop., 1903, p. 256; Brölemann, Cat. Myr. Brésil., 1909, p. 25.
(For synonymy and bibliography cf. Kraepelin, Loc. cit.).

Localities.—State of Bahia: Bahia! (Nathaniel Thayer expedition, M. C. Z.); State of Rio de Janeiro: Rio de Janeiro! (M. C. Z.); Brazil without definite locality (M. C. Z.); also recorded by Gervais as *audax*; by Newport as *gervaisi*, and *placeae*; by Koch, Kohlrausch, and Brölemann as *armata*).

GEOPHILOIDEA.

SCHENDYLIDAE.

Of this family, the largest of the Geophiloidea in the known fauna, three genera are found in Brazil, Schendylurus, Adenoschendyla, and Thalthybius (Ballophilini).

SCHENDYLURUS Silvestri.

Mitth. Naturh. mus. Hamburg, 1907, 24, p. 245; Brölemann et Ribaut, Bull. soc. ent. France, 1911, p. 192; Arch. Mus. hist. nat., 1912, ser. 5, 4, p. 113.
Schendyla Brölemann (ad. part. max.), Cat. Myr. Brésil, 1909, p. 6.

The Brazilian species known may be separated by means of the key. *Schendylurus brasiliensis* Silvestri, probably belonging to this genus, is not taken up, the published description being too meager to furnish sufficient information.

Key to Species.

- a. Pairs of legs less than fifty.
 - b. Ventral pores occurring on the first sternite; pores on each sternite divided into three areas; cephalic plate scarcely longer than wide.
 - Pairs of legs forty-one.
 - S. luderwaldi* Brölemann and Ribaut.
 - bb. No ventral pores on the first sternite; ventral pores, at least on the anterior sternites, in an undivided area; cephalic plate considerably longer than wide.
 - c. Antennae three times or more the length of the cephalic plate; last article of anal legs longer and conspicuously more slender than the penult; pairs of legs (♀) 47.
 - S. perditus*, sp. nov.
 - cc. Antennae but two times, or less, the length of the cephalic plate; last article of anal legs of nearly same length and thickness as the penult; pairs of legs (♀) thirty-seven.
 - S. bakeri*, sp. nov.
- aa. Pairs of legs near or above sixty.
 - b. Prebasal plate not exposed.....*S. gounellei* Brölemann.
 - bb. Prebasal plate exposed.....*S. paulista* Brölemann.

SCHENDYLURUS LUDERWALDI Brölemann and Ribaut.

Bull. Soc. ent. France, 1911, p. 220; Arch. Mus. hist. nat., 1912, ser. 5, 4, p. 117, fig. 48-52.

Locality.—State of Rio de Janeiro: Campo Itatiaya (Mus. Paul. coll.).

SCHENDYLURUS BAKERI, sp. nov.

Very pale; more densely pigmented anteriorly, where the color is pale lemon-yellow. Head and the prosternum with prehensors chestnut or with slight tendency to ferruginous. Antennae similar to head but lighter.

Body moderate; conspicuously narrowed from a little back of the middle caudad, but only moderately attenuated cephalad. Hairs of body and legs sparse, those of the legs chiefly toward the distal

ends of articles as usual, these also being longer than the more proximal ones.

Cephalic plate widest in front of middle where it bulges convexly on each side; sides of head caudad of this straight and a little converging to level of posterior end of first joint of prehensors (femur), then abruptly more strongly converging to the caudal corners which are not rounded; caudal margin straight; anteriorly the head is convexly widely rounded. Longer than wide, the ratio being nearly 43:38.

Antennae short being only 1.9— times longer than the cephalic plate; scarcely attenuated. Hairs very short, denser on the more distal articles, with hairs longer and more sparse on the proximal ones. Articles short, decreasing distad, with the sides more nearly straight than in *perditus*; ultimate article not much differing in length from the two preceding taken together.

Prebasal plate exposed.

Basal plate conspicuously narrowed cephalad; trapeziform. Two and a third times wider than long. Slightly more than one third as long as the cephalic plate (ratio 1:2.8–2.9).

Claws of prehensorial feet when closed attaining the front margin of the cephalic plate. Joints all unarmed within as usual. Sides of prosternum for most of length nearly straight and but slightly converging caudad, more abruptly rounding into caudal corners. Much wider than long, the ratio being 47:34. Longer than the first joint of prehensors in ratio 3:2.

Dorsal plates mostly showing a fine median sulcus in addition to the lateral ones. Anterior prescuta short, those of the middle and posterior regions becoming rather long, the last few then again short.

Spiracles all circular; the first considerably larger than the second, the others decreasing caudad and those of the posterior region very small or minute.

First fourteen or fifteen sternites angularly produced at middle of caudal margin, the process small; process fitting into an excavation in the succeeding segment as usual. The anterior margin of the second sternite conspicuously extended from sides to middle, that of the third segment similarly but less strongly produced, that of the fourth merely convexly bowed out, and those of the succeeding ones straight, or nearly so, or even a little incurved. Ventral pores present on all sternites excepting the first and the last; pore area subcircular, with the pores numerous. Sternites mostly showing a longitudinal median furrow which is deepest just in front of the middle, and a weaker transverse furrow which curves across in front of the pore area.

Last ventral plate very wide; trapeziform; the sides moderately converging caudad; caudal margin mesally incurved as in *manni* (Plate 4, fig. 7).

Coxopleural pores appearing as two large pits on each side, these being wholly covered by the last ventral plate excepting for a small ectal portion of each (Plate 4, fig. 7).

First pair of legs a little more slender than the second, but not at all or but slightly shorter. Posterior pairs of legs longer and proportionately more slender than the anterior ones.

Anal legs (♀) much longer than the penult. Scarcely thickened. The ultimate article about equal in length to the preceding one and not more slender; but the last two articles together more slender than the tibia. Hairs more numerous than on other legs, especially on the proximal joints (Plate 4, fig. 7). Pairs of legs (♀) forty-seven.

Length 24 mm.

Locality.—State of Amazonas: Manáos! (Mann and Baker).

One female.

Manifestly close in the main structural features to *S. perditus*. It is a materially larger form; has forty-seven pairs of legs as against thirty-seven in the latter species, has the antennae relatively much shorter; and the last article of the anal legs is proportionately much shorter and thicker as shown in the figures.

SCHENDYLURUS PERDITUS, sp. nov.

Body whitish, tinged with dilute lemon-yellow which is more evident anteriorly. Head and prosternum with prehensors ferruginous. Antennae brownish yellow of faint ferruginous tinge.

Moderate or slender; only slightly attenuated cephalad, more abruptly so caudad. Hair very sparse and mostly short over body, and those of legs also sparse.

Cephalic plate evidently longer than wide, the ratio being about 5:4.4. Widest toward anterior end where the sides are convex; from this region caudad the sides are more nearly straight and converge at first moderately and then more abruptly toward the posterior corners; caudal margin appearing considerably incurved. Frontal suture not present. (Plate 4, fig. 1).

Labral margin armed in the type with eighteen rather large, subacute, and strongly chitinous denticles, those at the sides being smaller, with apices turned mesad, and more acute than the more median ones.

First maxillae of usual structure; outer branch robust, without lappets.

Claw of palpus of second maxillae long, distally slender and acute and strongly curved; pectinate along both edges, the divisions long and slender.

Antennae moderately long, being three and a fifth times as long as the cephalic plate, only a little attenuated distad. Articles of proximal portion moderately long, each somewhat clavately widening from proximal and distad; the five articles preceding the ultimate short and relatively wider. Hairs of the last six or seven articles very short and rather numerous, on the more proximal articles becoming much more sparse and manifestly longer.

Prebasal plate exposed.

Basal plate trapeziform, the sides convex. About one third as long as the cephalic plate and 2.4-2.5 times wider than long.

Claws of the prehensors when closed not fully attaining the front margin of the head. Claws smooth. All articles unarmed within as usual.

Prosternum with sides for most of length nearly straight, a little converging caudad, more abruptly rounding mesad at posterior corners. Anterior margin well chitinized; but not at all denticulate; mesal incision between lateral portions shallow, semicircular; sloping from each side to the middle, there forming an obtuse reentrant angle. Prosternum much wider than long, the ratio being about 25:18; longer than the outer length of femur in about ratio 3:2. Hairs of prehensors sparse and in part moderately long; those of prosternum very sparse and short. (Plate 4, fig. 2).

Prescuta of the anterior and of the posterior fourths of length short, the others being moderately long. Sulci sharply impressed.

Spiracles all circular; the first conspicuously larger than the second, the others decreasing in size caudad and becoming very small or minute in the posterior region.

The more anterior sternites with a rather narrow angular median caudal process which fits into a corresponding excavation in the succeeding sternite. Each with a subcruciform impression which is considerably expanded in the region where the furrows cross. Pores beginning on the second segment where there are from forty to forty-five in the type; pores present on all succeeding sternites excepting the last, those of the penult segment being fewer in number; pores arranged in an undivided circular area.

Last ventral plate wide, trapeziform, the sides being nearly straight

and converging caudad; caudal margin angularly excised at middle, convex laterally toward and about each caudal corner. (Plate 4, fig. 3).

Each coxopleura with glands in the form of two large pits which are entirely simple and homogeneous; the anterior pore wholly and the posterior one mostly covered by the last ventral plate. (Plate 4, fig. 3).

First pair of legs a little more slender than the second but only slightly shorter. Posterior legs longer and proportionately more slender than the anterior ones. Anal legs much longer than the penult. Slender in the female. The distal joint somewhat longer than the preceding one and much more slender. Pairs of legs (♀) thirty-seven.

Locality.—State of Parahyba: Independencia! hills north of the town. (Mann and Heath).

SCHENDYLURUS GOUNELLEI (Brölemann).

Schendyla gounellei Brölemann, Ann. Soc. ent. France, 1902, **71**, p. 685; Cat. Myr. Brésil., 1909, p. 6.

Schendylurus gounellei Brölemann et Ribaut, Arch. Mus. hist. nat., 1912, ser. 5, **4**, p. 119, fig. 6, 62–67.

Locality.—State of São Paulo: Fazenda Nova Nicaragua.

SCHENDYLURUS PAULISTA (Brölemann).

Schendyla paulista Brölemann, Rev. Museu Paulista, 1903, **6**, p. 83, pl. 1, fig. 6–7; Cat. Myr. Brasil., 1909, p. 6.

Locality.—State of São Paulo: Poco Grande.

In many ways close to the preceding species and possibly but a variety of it.

(?) SCHENYDLURUS BRASILIANUS (Silvestri).

Nannophilus brasilianus Silvestri, Ann. soc. ent. Belg., 1907, **41**, p. 346.

Schendyla brasiliiana Brölemann, Cat. Myr. Brésil., 1909, p. 6.

Locality.—Brazil (precise locality not indicated).

The generic position of this species cannot be determined from the

original description. It appears not to be a true *Nannophilus* under which genus it was described, and is most probably a member of *Schendylurus*.

ADENOSCHENDYLA Brölemann and Ribaut.

Bull. Soc. ent. France, 1911, p. 192; Nouv. Arch. Mus. hist. nat., 1912, ser. 5, 4, p. 194.

Three species and one variety of this genus, which is peculiar to tropical and subtropical America, are known from Brazil. Of these, one is here first described.

The genus is close to *Pectiniunguis*. *Pectiniunguis* and *Adenoschendyla* lack claws on the anal legs in contrast with species of the southwestern United States (*montereus*, *heathi*, etc.) The latter species differ as well in other respects and may be placed under a distinct genus to be known as *Nyctunguis* (*P. montereus* Chamb., type).

Key to Species.

- a. Prebasal plate not exposed; none of the pore areas of the sternites are divided.....*A. plusiodonta* (Attems).
- aa. Prebasal plate exposed; some of the sternites of the median or posterior region of body longitudinally divided or geminate.
 - b. Head much longer than wide (ratio 4:3 to 4:3.5), pairs of legs fifty-three to fifty-nine.
 - c. Pores present on first sternite; head wider caudad than cephalad, longer than wide in ratio 4:3; pairs of legs 59 (♀); length 40 mm.....*A. parahybae*, sp. nov.
 - cc. No pores present on first sternite; head of same width anteriorly and posteriorly, longer than wide in ratio 4:3.5; pairs of legs 53 (♂)–55 (♀); length 25 mm.
 - A. geayi* Brölemann and Ribaut.
 - bb. Head but slightly longer than wide (ratio not more than 10:9), widest cephalad; pairs of legs forty-seven to fifty-three.
 - c. Claw of palpus of second maxillae of usual form.
 - A. imperfossa* (Brölemann).
 - cc. Claw of palpus of second maxillae globular at base.
 - A. imperfossa bolbonyx* Brölemann and Ribaut.

ADENOSCHENDYLA PARAHYBAE, sp. nov.

Mostly pale yellowish white, becoming more densely pigmented anteriorly, lemon-yellow. Head dilute ferruginous or orange, darker in a band immediately caudad of frontal region. Prosternum a little paler than the head, with the prehensors much lighter, yellow. Antennae yellow.

Rather slender with the body considerably attenuated cephalad and also very strongly at caudal end. Hairs sparse, of moderate length, more numerous caudad.

Cephalic plate much longer than wide (4:3). Widest caudad, conspicuously narrowed or constricted in frontal region at anterior end; anterior border subtriangular; caudal margin slightly concave; sides nearly straight from a little in front of caudal corners cephalad to frontal region. (Plate 5, fig. 1).

Antennae strictly filiform as usual. Long, being a little more than three times the length of the cephalic plate. Articles mostly long, excepting those immediately preceding the ultimate. Ultimate article longer than the two preceding taken together. Hairs of articles of distal region very short, dense; those of proximal articles conspicuously longer and more sparse.

Prebasal plate a little exposed, the cephalic plate not overlapping the basal.

Basal plate with sides straight, strongly converging cephalad; three times wider than long.

Claws of the prehensors when closed about even with the front margin of the cephalic plate; claws large and well overlapping; robust; articles all unarmed within as usual.

Prosternum wider than long in about ratio 7:5; one and a half times longer than the outer height of the femur of prehensors; sides converging from the anterior corners to the caudal and straight excepting towards ends; anterior margin sloping a little caudad of directly mesad from the ectal ends to the mesal incision, which is shallow, laterally strongly chitinized but with no signs of teeth.

Dorsal plates bisulcate; the sulci on the anterior plates distinct caudad as far as a fine transverse furrow a little in front of the caudal margin, this transverse furrow being in the form of a pair of concave impressions meeting at a cephalically directed angle; a fine median longitudinal sulcus also present as may also be one or two less distinct ones on each lateral part. Anterior prescuta short, the others increasing in length to the caudal region where they are moderately long, the last ones being again short.

Spiracles all circular; the first one much larger than the second and the latter likewise considerably larger than the third; the others gradually decreasing caudad, in the posterior region becoming minute.

The first sixteen sternites with the caudal border produced at the middle, the distinct process in each case fitting into a corresponding excavation in the succeeding segment. Ventral pores present on all sternites excepting the ultimate; on the sternite as far back as the twenty-fifth or twenty-sixth, the pores are in a single distinct sub-circular area; caudad of this the areas are more irregular, with a distinct tendency for each to become longitudinally divided into two areas or geminate.

Last ventral plate very wide; sides nearly straight, strongly converging caudad; caudal corners rounded; caudal margin a little crenately incised a little each side of the middle. Rather densely clothed with fine short hairs, especially on the caudal portion.

Coxopleurae subdensely clothed over the ventral area with fine short hairs.

First pair of legs shorter and more slender than the second, the next few pairs gradually attaining the full size; anterior pairs of legs conspicuously more robust than the posterior ones.

Anal legs in the female much longer than the penult; very slender. Ultimate joint longer than the penult, very slender and ending in a minute membranous point but with no trace of a real claw. Hairs long and sparse. (Plate 5, fig. 3). Pairs of legs fifty-nine.

Length 40 mm.

Locality.—State of Parahyba: Independencia! (Mann and Heath).

The present species differs from *plusiodonta* (Attems) in the much greater length and different shape of the cephalic plate, this in *plusiodonta* being only about as long as wide; in having the prebasal plate exposed; in the greater number of pairs of legs; in the character of the ventral pore areas, etc. The two species are similar in regard to the processes and pits of the anterior plates.

ADENOSCHENDYLA PLUSIODONTA (Attems).

Pectiniunguis plusiodontus Attems, Zool. jahrb. Syst., 1903, 18, p. 193, pl. 13, fig. 18; Chamberlin, Proc. Acad. nat. sci. Phil., 1904, p. 654.

Adenoschendyla plusiodonta Brölemann et Ribaut, Nouv. Arch. Mus. hist. nat., 1912, ser. 5, 4, p. 106.

Locality.—State of Santa Catherina: Blumenau.

ADENOSCHENDYLA IMPERFOSSA (Brölemann).

Schendyla imperfossa Brölemann, Rev. Mus. Paulista, 1901, 5, p. 44, pl. 1, fig. 8-13; Cat. Myr. Brésil, 1909, p. 6.

Adenoschendyla imperfossa Brölemann et Ribaut, Nouv. Arch. Mus. hist. nat. 1912, ser. 5, 4, p. 107.

Locality.— Brazil. (Museu Paulista).

ADENOSCHENDYLA IMPERFOSSA BOLBONYX Brölemann and Ribaut.

Brölemann et Ribaut, Nouv. Arch. Mus. hist. nat., 1912, ser. 5, 4, p. 107, fig. 18-23.

Locality.— State of São Paulo (type Museu Paulista).

ADENOSCHENDYLA GEAYI Brölemann and Ribaut.

Bull. Soc. ent. France, 1911, p. 219; Nouv. arch. Mus. hist. nat., 1912, ser. 5, 4, p. 108, pl. 2, fig. 24-30, pl. 3, fig. 31-32.

Locality.— State of Amazonas (Brazilian Guiana, Lower Carsevenne or Calçoene, Geay, collector).

THALTHYBIUS Attems.

Zool. jahrb. Syst., 1900, 13, p. 139; 1903, 18, p. 183.

PRIONOTHALTHYBIUS Brölemann.

Arch. zool. exp. et gen., 1909, ser. 5, 3, p. 334.

THALTHYBIUS (PRIONOTHALTHYBIUS) PERRIERI Brölemann.

Bull. Mus. hist. nat., 1909, p. 1, fig. 8-10.

Locality.— State of Amazonas (Brazilian Guiana, Upper Carsevenne or Calçoene; Geay, collector).

Of Uncertain Position.(?) *GEOPHILUS SUBLAEVIS* Meinert.

Natur. tidskr., 1870, ser. 3, 7, p. 72.

Locality.— State of Minas Geraes: Lagoa Santa.

This is certainly not a true *Geophilus*, being in all probability a member of the present family. It seems likely to prove to belong to *Schendylurus*. The anal legs are unarmed; the last ventral plate very wide with pores on coxopleurae said to be absent by Meinert but no doubt to be found after proper treatment with potash as has been shown to be true with various species of *Schendylurus*, etc., which at first were considered to lack the pores; the prosternum and joints of prehensors, unarmed, the claws of the latter not surpassing the front margin of the head; pairs of legs 67.

ORYIDAE.

ORPHNAEUS Meinert.

Myr. Mus. Hauniensis, 1870, 1, p. 17; Proc. Amer. philos. soc., 1886, 23, p. 230; Zool. jahrb. Syst., 1903, 18, p. 200; Verhoeff. Bronn's Thierreich, 1908, 5, p. 294.

Chomatobius Humbert et Saussure, Rev. mag. zool., 1870, p. 205; Miss. scient. Mex., 1872, p. 145.

ORPHNAEUS BRANNERI, sp. nov.

Dorsum yellowish brown, darker cephalad where the tergites are margined with a more deeply red stripe. The anterior dorsal plates, excepting the first one, with a conspicuous black spot on the anterior portion, this consisting typically of a narrow transverse stripe along the anterior margin connected at the middle by means of a broad neck with two short curved marks diverging from each other and bending out laterad near the middle of the plate; this mark in going from segment to segment caudad becoming less and less developed and finally disappearing entirely. No distinct geminate dark stripe such

as is so characteristic of *brevilabiatus*. Basal and cephalic plates deep ferruginous; prosternum and prehensors ventrally similar but paler, the claws black. Antennae like cephalic plate but pale at their very tips. Venter pale testaceous, darker cephalad. Legs similar to venter.

Body large and robust; strongly attenuated both cephalad and caudad. Hairs of body very fine and short as are also the few hairs of the legs.

Cephalic plate conspicuously wider than long, the ratio being about 48:39. Widest caudad, where the sides are convex; moderately converging anteriorly in front of the middle to the anterior corners; lateral portions of anterior margin converging from the anterolateral corners to an obtuse angle at the middle; caudal margin widely, weakly convex. Plate with subdense, uniform, fine punctae. Hairs very fine and short, numerous.

First maxillae with inner division sharply set off; short and broad, apically rounded, not membranous. Outer division with the second and third articles not separated by a suture; short and thick; slightly membranous at tip on mesal side; membranous lappets of moderate length, the distal one wide and rather dorsal in position. Coxae of the second maxillae broadly joined at middle; with the usual oval opening toward the caudal end of each side. Claw of palpus rather small, bearing along each edge a fringe of about nine or ten spines.

Antennae very short, being but 1.68 times the length of the cephalic plate. Flattened; very wide at base, then strongly narrowed, especially distad of about the proximal fourth. Proximal articles very short, much wider than long, the more distal ones relatively longer; the ultimate article not much differing in length from that of the two preceding ones taken together. Hairs very fine and short, dense distad, becoming less so proximad.

Prebasal plate not exposed.

Basal plate embracing the cephalic. Very wide, with sides convex and not strongly converging cephalad; nearly one half as long as the cephalic plate (ratio about 2.1:1), and very nearly three times as wide as long; finely and subdensely punctate like the head. Hairs similar to those of the head but considerably fewer in number.

Claws of the prehensors when closed very nearly but not wholly attaining the front margin of the cephalic plate; all joints unarmed; claws stout.

Prosternum much wider than long, the ratio of width to length being 2.25-2.3:1; longer than the outer height of femur in ratio 25:14; subdensely punctate as are also the proximal articles of the prehensors. (Plate 5, fig. 5).

Dorsal plates with a pair of longitudinal deeply impressed sulci on the middle portion and an additional longitudinal sulcus farther laterad on each side less sharply impressed; indications on some segments also of a shallow median longitudinal median furrow. First dorsal plate considerably wider than the second and at its ends bent ventrad toward the base of each leg and crenately incised on the caudal side near each corner (Plate 5, fig. 4).

Spiracles large; all elliptic, the anterior ones being oblique but more nearly horizontal than vertical, becoming strictly longitudinal caudad; first spiracle not larger than the second; those of the caudal region smaller as usual. Suprascutella large and distinct in the posterior region but absent in the anterior.

Ventral pores in two broad transverse bands connected at the ends and thus forming a quadrangle. Enclosed area mostly with a distinct transverse furrow or row of impressed spots or the whole area roughened with irregular impressions, in most more deeply impressed longitudinally at middle.

Last ventral plate pentagonal, the sides strongly converging caudad and the caudal margin straight. Marked with a longitudinal median furrow which is not especially deep.

Coxopleurae rather large, coxiform; longer than thick in the direction of thinnest diameter; the trochanter only about one third as long.

Anal legs in male considerably shorter than the penult; composed of six articles¹ which decrease in diameter regularly from base distad. Pairs of legs 77.

Length 88 mm.; length of antennae, 2.2 mm.; greatest width of body, 3.3 mm.; width of first dorsal plate 1.9 mm.

Locality.—State of Rio Grande do Norte: Natal! (Mann).

While this species is close in many features to *brevilabiatus*, it is very easily separated from this wide-spread form. It is most readily distinguished by the antennae which are much shorter, extending only to the caudal end of the basal plate or thereabouts, whereas in *brevilabiatus* they reach upon or toward the caudal end of the second pediferous segment; also the antennae are conspicuously wider at the base and more strongly attenuated (Plate 5, fig. 4). The dorsal plate of the first segment is clearly different, being bent farther ventrad of ends and being more considerably notched on caudal side toward each caudolateral corner. It lacks the conspicuous geminate dorsal black stripe so characteristic of *brevilabiatus*.

¹ The right leg of the type specimen appears to have been regenerated. It is shorter than the other and consists of but five articles.

ORPHNAEUS BREVILABIATUS (Newport).

Geophilus brevilabiatus Newport, Trans. Linn. soc. London, 1844, 19, p. 436.
Geophilus lineatus Newport, Ibid.

Geophilus guillemini Gervais, Insect. Aptères, 1847, 4, p. 311.

Chomatobius brasilianus Humbert et Saussure, Rev. mag. zool., 1870, p. 205;
 Miss. scient. Mex., 1872, p. 146, pl. 6, fig. 24.

Orphnaeus brasiliensis Meinert, Myr. Mus. Hauniensis, 1870, 1, p. 20; Proc.
 Amer. philos. soc., 1886, 23, p. 232; Bollman, Proc. U. S. nat. mus., 1888,
 11, p. 337; Brölemann, Mem. Soc. Zool. France, 1900, 13, p. 92; Ann.
 Soc. ent. France, 1902, 71, p. 652; Zool. anz., 1903, 26, p. 178; Rev. Mus.
 Paulista, 1903, 6, p. 71; Attems, Zool. jahrb. Syst., 1903, 18, p. 201; Cat.
 Myr. Brésil, 1909, p. 5.

Orphnaeus brevilabiatus Pocock, Journ. Linn. soc. London, 1893, 24, p. 472;
 Biol. Centr. Amer. Chilopoda, 1895, p. 40.

Orphnaeus brasilianus nigropictus Attems, Loc. cit., p. 203.

Localities.— State of Rio Grande do Norte: Ceará-Mirim! (Mann and Heath); State of Parahyba: Independencia! (Mann and Heath); State of Amazonas: Manáos; State of Pará: Pará; State of Pernambuco: Pernambuco; State of Rio de Janeiro: Rio de Janeiro!.

NOTIPHILIDES Latzel.

Myr. Öst-Ung. monarch., 1880, 1, p. 20; Zool. anz., 1880, 3, p. 546; Meinert, Proc. Amer. phil. soc., 1886, 23, p. 233; Attems, Zool. jahrb. Syst., 1903, 18, p. 233; Verhoeff, Bronn's Thierreich, 1908, 5, p. 292.

NOTIPHILIDES GRANDIS Brölemann.

Rev. Mus. Paulista, 1903, 6, p. 71, pl. 1, fig. 8-11.

Locality.— State of Amazonas: Manáos.

It was possibly a specimen of this species to which Cook gave the name *Heniorya longissima*; but as no description of the species is given both the generic and specific names, as Brölemann justly suggests, stand purely as *nomina nuda*.

APHILODONTIDAE.

MECISTAUCHENUS Brölemann.

Brasilophilus Brölemann, Bull. Soc. ent. France, 1907, p. 283. Verhoeff, Bronn's Thierreich, 1908, 5, p. 286.

MECISTAUCHENUS MICRONYX Brölemann.

Aphilodon micronyx Brölemann, Rev. Mus. Paulista, 1901, **5**, p. 46; Cat. Myr. Brésil., 1908, p. 3.

Mecistauchenus micronyx Brölemann, Bull. Soc. ent. France, 1907, p. 283.

Locality.— Brazil (precise locality not reported).

APHILODON Silvestri.

Comm. Mus. nac. Buenos Aires, 1898, **1**, p. 39; Attem, Zool. jahrb. Syst., 1903, **18**, p. 215, 283; Verhoeff, Bronn's Thierreich, **5**, p. 279, 282; Silvestri, Boll. Lab. zool. R. sc. Agric. Portici, 1909, **4**, p. 53.

APHILODON ANGUSTATUS Silvestri.

Rend. R. accad. Lincei, ser. 5, **18**, p. 269; Boll. Lab. zool. R. sc. Agric. Portici, 1909, **4**, p. 56.

Locality.— State of Matto Grosso: Urucum, Corumbá. Also reported from Paraguay and Argentina.

MECOPHILUS Silvestri.

Rend. R. accad. Lincei, 1909, ser. 5, **1**, **18**, p. 268.

MECOPHILUS NEOTROPICUS Silvestri.

Rend. R. accad. Lincei, 1909, ser. 5, **1**, **18**, p. 269.

Locality.— State of Paraná: Iguassú.

MECISTOCEPHALIDAE.**MECISTOCEPHALUS** Newport.

Proc. Zool. soc. London, 1842, p. 178; Trans. Linn. soc. London, 1844, **19**, p. 276; Meinert, Naturh. tidsskr., 1870, ser. 3, **7**, p. 92; Latzel, Myr. Ost-Ung. monarch., 1880, **1**, p. 160; Meinert, Proc. Amer. philos. soc., 1886, **23**, p. 212; Haase, Abhandl. Mus. Dresden, 1887, **5**, p. 100.

Lamnonyx Attems, Zool. jahrb. Syst., 1903, **18**, p. 210; Verhoeff, Bronn's Thierreich, 1908, **5**, p. 273.

MECISTOCEPHALUS PUNCTIFRONS Newport.

Proc. Zool. soc. London, 1842, p. 179; Trans. Linn. soc. London, 1844, 19, p. 429; Meinert, Naturh. tidskr., 1870, ser. 3, 7, p. 97; Chamberlin, Ent. news, 1913, p. 122.
Mecistocephalus guildingi Newport, Loc. cit., p. 429; Meinert, Loc. cit., p. 96; Latzel, Mitt. Mus. Hamburg, 1895, 12, p. 101; Pocock, Trans. Linn. soc. London, 1893, 24, p. 470; Attems, Zool. jahrb. Syst., 1903, 18, p. 209.
Mecistocephalus sulcicollis Tömösvary, Termes, füzetek, 1885, 5, p. 64.
Lamnonyx punctifrons Attems, Loc. cit., p. 211.

Locality.— State of Amazonas: Manáos! (Mann and Baker).

This appears to be the first record of the occurrence of a member of the Mecistocephalidae in Brazil. The species is common in the Bermudas and West Indies. It must logically be regarded as the type of *Mecistocephalus* proper, the other species originally included under this name by Newport having been removed to other genera. It would seem that Newport did not at the time he erected the genus, know or have in hand any species congeneric with *carniolensis* and that in consequence there appears no justifiable way of continuing the prevalent practice of applying the name *Mecistocephalus* to the genus including these species.

As no difference of distinctive value has been pointed out between *punctifrons* and *guildingii* and as different authors refer to American specimens at times under one and at other times under the other name, I have united the two as one species as was long ago suggested by Meinert. If the form occurring in the western hemisphere shall be found to differ definibly from that of the eastern hemisphere, it must bear the name *guildingii*.

TYGARRUP, gen. nov.

Body widest near middle, attenuated moderately cephalad and more strongly caudad.

Head large. Cephalic plate longer than wide, narrowed caudad. Frontal suture present.

Antennae long, filiform.

Clypeus proper large, triangularly extending forward in middle to between antennae, at middle being three times greatest length of labrum; clothed with few hairs.

Labrum tripartite, the median piece narrow and caudally one-

toothed; the lateral pieces smooth, not longitudinally striate; not much bowed.

From each anteroectal corner of labrum a suture extends obliquely cephaloectad, separating the median from the lateral divisions of the ventral portion of the cephalic plate; lateral division narrowed caudad, the mesal edge strongly chitinized and extended cephalad into an angular process as in related genera.

Mandibles with pectinate lamellae only.

First maxillae with coxae completely separated, though closely appressed at median line. Inner branch clearly separated from coxa; subtriangular; distally prolonged into a conspicuous membranous lobe which is nearly as long as the proximal portion. Outer branch with second and third divisions completely coalesced; narrow; extended distally into a long membranous lobe like that of inner division; no lappets present. Second maxillae with coxae rather short; coalesced at median line but more narrowly than in *Mecistocephalus*, etc. Pore of salivary gland on ectal portion of coxosternum near middle of length, not at caudal angle, the passage extending ectad to lateral margin. Palpi terminating in short, nearly straight, claws.

Prehensors large, much exposed from above.

Claws extending beyond front margin of head. Some of joints mesally armed.

Basal plate narrow.

Prebasal plate not exposed. Pleurae exposed at sides of basal plate.

Dorsal plates bisulcate.

Ventral pores absent.

Last ventral plate subtriangular. Coxopleurae large, porose.

Anal legs with six joints distad of coxopleurae; clawless.

Anal pores present.

Type.— *T. intermedius*, sp. nov.

This genus is most closely related, apparently, to that embracing *carniolensis*, *limatus*, etc. (*Mecistocephalus* of most authors). It is different chiefly in the following points:—the materially greater shortness of the coxopleurae of the second maxillae and particularly the difference in position of the salivary pore, this being at about middle of length and toward lateral margin, not at extreme caudal angle as in *Mecistocephalus*, etc.; and the lateral divisions of the labrum being unarmed, that is smooth, and not longitudinally striate. Also the hairs of labrum are much more sparse. From *Mecistocephalus* proper (*punctifrons* Newport, etc.) it may readily be separated through the absence of the strongly chitinized process or tooth on the

ventral border of head at anteroectal end of lateral division and the much shorter coxosternum of the second maxillae and difference in position of pore; the smaller middle piece of the labrum; and the larger clypeus, which extends cephalad in triangular form to near level of antennae. It agrees with the latter genus in the unarmed character of the lateral pieces of the labrum and also in the large size of the membranous lobe of the inner division of the first maxillae.

TYGARRUP INTERMEDIUS, sp. nov.

Yellowish; in type with a pale median longitudinal line paralleled or limited on each side by a somewhat darker stripe which is deepest cephalad and caudad, but these not evident in younger specimens. Head and prosternum with prehensors pale ferruginous. Antennae and legs pale.

Head widest at level of labrum, conspicuously narrowed caudad with posterior corners well rounded; caudal margin straight; sides oblique and somewhat incurved from ends of frontal suture to ectal side of base of antennae; anterior margin substraight, narrowly semi-circularly excised at middle. Longer than wide in ratio 100:73. Pleural piece of ventral side of head plate with mesal edge strongly chitinized and ending cephalad in a pointed process as usual but with no trace of a tooth at cephaloectal angle. Clypeal region with hairs very sparse. Hairs of dorsal surface small and sparse.

Labrum with the median piece very narrow, the sides of this being for most of length nearly parallel or but little converging caudad, its caudal end narrowing to a subacute tooth. Greatest length of labrum (*i. e.* at ends) about one third the median length of the clypeal area.

Inner branch of the first maxillae with basal portion subtriangular in outline; the membranous distal division as long as or nearly as long as the basal, widening distad with mesal side concave and the ectal convex. Outer branch narrow, subcylindric, narrowing but moderately to the beginning of the long membranous distal division which is as long as the proximal division and widens distad like that of the inner division which it overtops by a short distance.

Coxosternum of second maxillae rather short; median portion membranous; mesocaudal portion also membranous and not sharply defined. Pore close to outer edge, to which a passage from it leads, and a little caudad of middle of length, five or six bristles forming a row parallel with and a little removed from the anterior margin on each

side. Claw of palpus small and pale, nearly straight, with a denticle on mesal side toward base.

Antennae nearly 2.4 times longer than the head. Articles moderate in length, decreasing very gradually distad. Ultimate article (in mature specimen) shorter than the two preceding taken together in about the ratio 3:4, in young specimens longer than these two.

Claws of the prehensors when closed reaching to the end of the first antennal segment. Claw without a true tooth within, there being, however, a slight low, rounded, chitinous elevation; intermediate joints unarmed; prefemur (femuroid) at distal end on mesal side with a distally rounded tooth projecting cephalomesad.

Prosternum a little wider cephalad than caudad; 1.3 times longer than wide; 1.9 times as long as length of prefemur on ectal side. Prosternum bearing on anterior margin each side of mesal incision a basally broad, conical tooth. Mesal incision with sides almost parallel, rounded at bottom. Basal plate a little overlapped both by head and by first dorsal plate; twice as wide as long; ratio of width at caudal end to that at anterior end as 45:34; head about 4.5 times longer.

Prescuta of posterior and median region short; those of anterior region very short.

Anterior ventral plates with a deep median longitudinal sulcus on caudal part and ending at about middle of plate in the angle of a short v or u-shaped impression, the arms of which diverge cephalad. This median sulcus becomes gradually weaker caudad, fading out and disappearing near the twenty-first segment, the u-shaped impression disappearing farther cephalad.

Spiracles circular, rather large; first one largest, with the third considerably smaller and the second intermediate, the other decreasing gradually caudad as usual.

First pair of legs greatly reduced, being only about two thirds the length of the second ones and much more slender. Anterior and posterior pairs not sensibly differing in length or thickness.

Last ventral plate triangular or shield-shaped, the sides being convex; narrowly truncate at caudal apex.

Coxopleurae moderately enlarged; each with two large pores partially covered by the ventral plate and over free ventral and lateral surface with regularly spaced, moderately numerous, smaller pores but these larger and fewer than those of *Mecistocephalus*, etc.

Anal legs much longer than the penult but proportionately slender. Hair moderate in size, subspare. Ultimate article narrowed distad, terminating in an obscure membranous tip.

Length of type, cir. 18 mm.

Locality.— British Guiana (taken at Washington, D. C., in pots of plants imported from that country).

One adult, or nearly adult, and three adolescent specimens.

GEOPHILIDAE.

The new genus and species described below, and *Ribautia bouvieri* Brölemann from the Carsevenne are the only representatives of this family, in the strict sense, at present known to occur within Brazil. Both genera belong to Chilenophilinae. Here also belongs *Taiyuna*, of which a representative from British Guiana is described.

SCHIZONAMPA, gen. nov.

Frontal suture not evident. Basal plate wide; overlapped by the cephalic plate. Antennae filiform. Dorsal plates bisulcate.

Labrum free; tripartite; the median piece of moderate size, triangular, with the free edge armed with teeth; lateral pieces fringed with more slender, spinescent processes.

Outer process of first maxillae uniarticulate; bearing well-developed membranous lappets. Inner branch seemingly set off by suture; Coxae fused at middle.

Second maxillae with coxae almost completely separated at middle, the connection being narrow and membranous. Pleurosternal sutures strongly developed. Palpus triarticulate, terminating in a large simple claw. Femur bearing at distoectal corner a strongly chitinized acute process; tibia also bearing a similar process in a nearly corresponding, or slightly more dorsal position. (Plate 6, fig. 6).

Prehensorial feet large, conspicuously exposed from above, and extending well beyond the front margin of the head; dentate within.

Prosternum without distinctly developed chitinous lines. Basal plate trapeziform; wide.

Ventral pores not evident.

Last ventral plate wide. Coxopleural pores appearing as two large pits on each side.

Anal pores not evident (in type).

Anal legs with seven joints distad of the coxopleura, the small

terminal article not bearing a claw. The extra article of the anal legs is at least strongly simulated on all the other legs, but especially the more posterior ones, by a contracted terminal division of the tarsus which for the most part is clearly distinct. (Plate 6, fig. 7).

Genotype.—*Schizonampa manni*, sp. nov.

This interesting genus is the second of the Chilenophilinae to be reported from Brazil, Ribautia being the first. Taiyuna, recorded from British Guiana, is the only other representative of the group at present known from South America.

Schizonampa may readily be separated from the other known genera of the Chilenophilinae lacking a claw on the anal legs and having the small additional distal article as shown in the following key.

Key to Genera of Section embracing Schizonampa.

- a. Ventral pores present; distomesal angle of coxa of second maxillae prolonged; first maxillae without lappets.
Proschizotaenia Silvestri.
- aa. Ventral pores absent; distomesal angle of coxa of second maxillae not prolonged; first maxillae with lappets.
 - b. Pores occurring as two large pits on each coxopleura; femur and tibia of second maxillae prolonged into an acute, strongly chitinized process at distoectal angle. . . *Schizonampa*, gen. nov.
 - bb. Coxopleural pores small and isolated; tibia not prolonged at distoectal angle.
 - c. First maxillae with two long membranous lappets on each side. *Watophilus* Chamberlin.
 - cc. First maxillae with but a single lappet on each side, this being borne on the femur. . . . *Alloschizotaenia* Brölemann.

SCHIZONAMPA MANNI, sp. nov. .

Slender; sides of body nearly parallel over most of length, but conspicuously attenuated at caudal end and moderately attenuated toward head. Body sparsely hirsute with short hairs; hairs of legs few, those present commonly arranged mostly toward the distal ends of articles.

Color of body very pale, whitish yellow, the yellow being very dilute. Head with basal plate, prosternum, and prehensors, darker, somewhat light orange or dilute ferruginous; the antennae also similar.

Cephalic plate much longer than wide (ratio about 4:29) narrowest cephalad; a little constricted in front of region where frontal suture would be if present, between which level and the caudal corners the sides are substraight or only very slightly convex; hairs sparse and mostly short or very short. Frontal plate not discrete. (Plate 6, fig. 1).

Antennae short, being only 1.9 longer than the cephalic plate; attenuated. Articles mostly short, decreasing in size distad, those between the fifth and the ultimate being especially short; ultimate article longer than the two preceding taken together. Hairs on the first four or five articles moderate in length, sparse, those of the more distal articles considerably shorter and more dense. (Plate 6, fig. 1).

Basal plate trapeziform as usual; much overlapped by the cephalic plate as also by the first succeeding tergite. Exposed portion very short, at the median line being but one eighth as long as the cephalic plate and being about 4.5 or 4.6 times wider than long. (Plate 6, fig. 1, 2).

Clypeal region without any porose area; areolae distinct and uniform excepting for a median area on the anterior portion in which the areolae are conspicuously reduced in size and on which four hairs are borne, the clypeal region being elsewhere glabrous.

Median piece of labrum rather large, triangular, bearing along the free margin five large acute and strongly chitinized teeth; lateral pieces with a fringe or more numerous slender spinescent processes. (Plate 6, fig. 4).

First maxillae bearing ectally on each side one moderately long membranous lappet. Coxosternum mesally incised, but the coxae well fused for most of length of contact. (Plate 6, fig. 5).

Coxae of second maxillae almost completely separated, there being only caudally a pale membranous connecting bridge. Pleurosternal sutures strongly developed. Coxa not at all produced at mesodistal angle. Femur and tibia bearing at distoectal angles a distinct, acute, well chitinized process, that of the tibia being somewhat more dorsal in position than that of the femur. Claw large and simple. (Plate 6, fig. 6).

Claws of the prehensor when closed extending much beyond the anterior margin of the cephalic plate; attaining the distal end of the second antennal article. Claw not crenulate; armed at base with a stout tooth which is subtruncate distally. Intermediate articles without trace of teeth, but the femur bearing on mesal side toward

distal end a stout, bluntly rounded, tooth and also bulging in a small well-rounded eminence near the proximal third where there is indication of a suture such as is frequently present (apparently of trochanter).

Lateral margin of prosternum parallel for most of their length, incurving only toward caudal ends. Prosternum a very little wider than long (ratio cir. 18:17); longer than the femur on outer side in about ratio 17:11; anterior margin bearing two low and rounded, strongly chitinous, teeth; hairs very sparse. (Plate 6, fig. 3).

The paired sulci of the tergites distinct; in addition to these a median longitudinal sulcus may be evident in the anterior region. Prescuta very short in the anterior region, becoming moderately long in the median and posterior regions. Hairs mostly very short.

Spiracles all circular. The first one distinctly larger than the second, the others gradually decreasing caudad as usual.

First pair of legs reduced, being shorter and decidedly more slender than the second. Posterior pairs of legs relatively but very little more slender than the anterior ones. A small third tarsal division simulating or corresponding to the extra one of the anal legs is evident on all legs but especially the more posterior pairs; it is short and considerably more slender than the preceding one.

Anterior ventral plates with a rather deeply impressed median longitudinal sulcus which extends entirely across the plate. First ten or eleven sternites produced caudad into a wide triangular process or lobe which fits into a recess in the anterior border of the succeeding plate in each case. Ventral pores not detected.

Last ventral plate very wide; trapeziform, the sides slightly convex anteriorly but substraight for most of length, strongly converging caudad; caudal margin straight. (Plate 6, fig. 7).

Coxopleural pores consisting of two large pits on each side; of these pits the anterior one each side is wholly covered by the ventral plate and the caudal one is covered excepting for a small portion. (Plate 6, fig. 7).

Anal legs much longer than the penult; slender in the female. Second joint of tarsus long and slender; the third very short and abruptly more narrow. (Plate 6, fig. 7). Pairs of legs in the type thirty-seven.

Length 13 mm.

Locality.—State of Pará: Pará! (Mann and Baker).

One female specimen was secured.

RIBAUTIA Brölemann.

Arch. zool. exp. et gen., 1909, ser. 5, 3, p. 335.

RIBAUTIA BOUVIERI Brölemann:

Bull. Mus. hist. nat., 1909, p. 7, fig. 19-26.

Locality.—State of Amazonas: Brazilian Guiana, on the upper Carsevenne or Calçoene (Geay, collector).

TAIYUNA Chamberlin.

Pomona college jour. ent., 1912, 4, p. 661.

This genus was previously known only from California and Arizona in which states three species are known to occur.

TAIYUNA AUSTRALIS, sp. nov.

Color yellow, of weak orange tinge cephalad. Head and prehensors darker, brown. Antennae similar to head, but paler distad.

Body attenuated cephalad, more strongly so caudad; moderately robust.

Head widest a little caudad of level of labrum from where the sides converge a little and are straight to the rounded posterior corners and also converge slightly cephalad to the similarly rounded anterior corners; posterior margin widely, somewhat flatly, convex; anterior margin with each side straight, extending from corner a little cephalad of mesad to the middle where there is a distinct notch. In type the head is 1.38+ times longer than wide.

Inner branch of first maxilla terminating in an auriculiform membranous lobe at distoectal corner; bearing 3 to 5 bristles. Distal joint of outer branch long, apically rounded with the dorsoectal edge strongly chitinized; bearing about six bristles; membranous lappets long and spinulose, the distal one larger than the proximal, and extending much beyond distal end of the outer branch.

Coxae of second maxillae united at middle merely by a narrow membranous bridge; more strongly chitinized along edge below disto-mesal angle at which there is no trace of a process. Pleurosternal

suture strongly marked as usual; salivary pore opening through the mesal border toward the anterior end in the usual way. Joints of palpus all without processes; femuroid narrowed proximally; claw small, simple.

Antennae short, 1.8 times as long as head; attenuated. Articles decreasing uniformly in length from the first to the penult; ultimate article about equal in length to the two preceding ones taken together. Hairs of proximal articles sparse, distally becoming shorter and finer and more dense.

Claws of prehensorial feet when closed reaching to or a little beyond distal end of first antennal joint; stout. Claw armed at base with a small, distally rounded tooth. Intermediate joints with slight, low and broad, chitinous denticles. First joint toward distal end and a little proximad of the corner with a thick rounded tooth.

Anterior margin of prosternum unarmed; mesal incision very slight, semicircular. Prosternum wider than long in ratio 64:59; 1.64 times as long as outer length of prefemur; sides straight, only slightly converging from anterior end to the rounded caudal corners.

Basal plate trapeziform, strongly narrowed cephalad; sides slightly convex caudad and slightly concave cephalad. 2.9 times as wide as median length, $\frac{1}{4}$ as long as head. Overlapped both by cephalic and by first dorsal plate; the length of exposed portion to total length inclusive of covered ends as 3:4. Plate as a whole about 1.85 times wider than long.

Anterior prescuta short, those of middle region becoming long and exceeding half the length of the major scuta, those of caudal region again short. Hairs short and sparse.

Eight or ten of the first ventral plates with a triangular lobe on caudal border fitting into an excavation in the succeeding plate. Plates smooth, without pronounced furrows, or in some showing a shallow median longitudinal depression.

Spiracles large, circular, or with the anterior ones very slightly longer dorsoventrally. First spiracle much exceeding the second in size, the others gradually decreasing caudad.

Legs of first pair a little more slender than the second, only slightly shorter. Anterior and posterior pairs not at all or but little differing in length and robustness.

Last ventral plate narrow; sides at first but slightly converging caudad, but more decidedly so toward posterior corners; caudal margin subtruncate. Depressed along the median longitudinal line.

Coxopleurae moderately inflated; ventrally pierced by about 14

or 16 pores, part of which are arranged along and partly covered by the last ventral plate; a pore somewhat larger than the others is isolated midway between the most caudal of the other pores and the distal end of coxopleura.

Anal legs moderately long; slender, the joints decreasing in diameter from the femur distad; second tarsal joint moderately attenuated distad and bearing a very small and slender but distinct claw.

Length about 42 mm.

Locality.—British Guiana. (Taken at Washington, D. C., in pots of plants imported from that country).

LITHOBIOMORPHA.

LITHOBIIDAE.

LITHOBIUS Leach.

Trans. Linn. soc. London, 1814, **11**, p. 381; Latzel, Myr. Ost-Ung. monarch., 1880, **1**, p. 31; Meinert, Proc. Amer. philos. soc., 1886, **23**, p. 174; Verhoeff, Bronn's Thierreich, 1907, **5**, p. 239.

LITHOBIUS FORFICATUS (Linné).

Scolopendra forficata Linné, Syst. nat. ed. 10, 1758, **1**, p. 638.

Lithobius forficatus Stuxberg, Ofvers. Kong. vet. akad. Forh., 1875, p. 27; Fedrizzi, Atti Soc. Ven-Trenk., 1875, **6**, p. 205; Latzel, Myr. Ost-Ung. monarch., 1880, **1**, p. 57; Meinert, Proc. Amer. philos. soc., 1886, **23**, p. 176.

Lithobius parvulus Fedrizzi, Loc. cit., p. 213.

Lithobius trilineatus Brölemann, Cat. Myr. Brésil., 1908, p. 33.

(For extended synonymy and bibliography cf. Stuxberg, Loc. cit.).

Locality.—Recorded from Brazil as *Lithobius trilineatus*, the type being the only record of the species or genus from the country. It was probably introduced.

SCUTIGEROMORPHA.

SCUTIGERIDAE.

PSELLIOPHORA Verhoeff.

Sitz. Gesellsch. nat. freunde Berlin, 1904, p. 259; Bronn's Thierreich, 1907, **5**, p. 230.

PSELLIOPHORA NIGROVITTATA (Meinert).

Scutigera nigrovittata Meinert, Proc. Amer. philos. soc., 1886, **23**, p. 173; Pocock, Biol. Centr. Amer., 1895, p. 650; Brölemann, Ann. Soc. ent. France, 1902, **71**, p. 650; Brölemann, Cat. Myr. Brésil, 1908, p. 34.

Localities.— State of Rio Grande do Norte: Natal! (W. M. Mann. Numerous specimens); State of Parahyba: Independencia! (Mann and Heath); State of Matto Grosso: Madeira-Mamore R. R. camp 41, on the Rio Madeira! (W. M. Mann); State of Bahia: Santo Antonio da Barra); Also Brazil, without special locality (M. C. Z.).

PLATE 1.

Cryptops heathi Chamberlin.

1. Dorsal view of head and first dorsal plate.
2. Prosternum and prehensors.
3. Anal leg.

Mimops occidentalis, Chamberlin.

4. Dorsal view of head and first dorsal plate.
5. Prosternum and prehensorial feet.
6. Last dorsal plate.
7. Last ventral plate and coxopleurae.

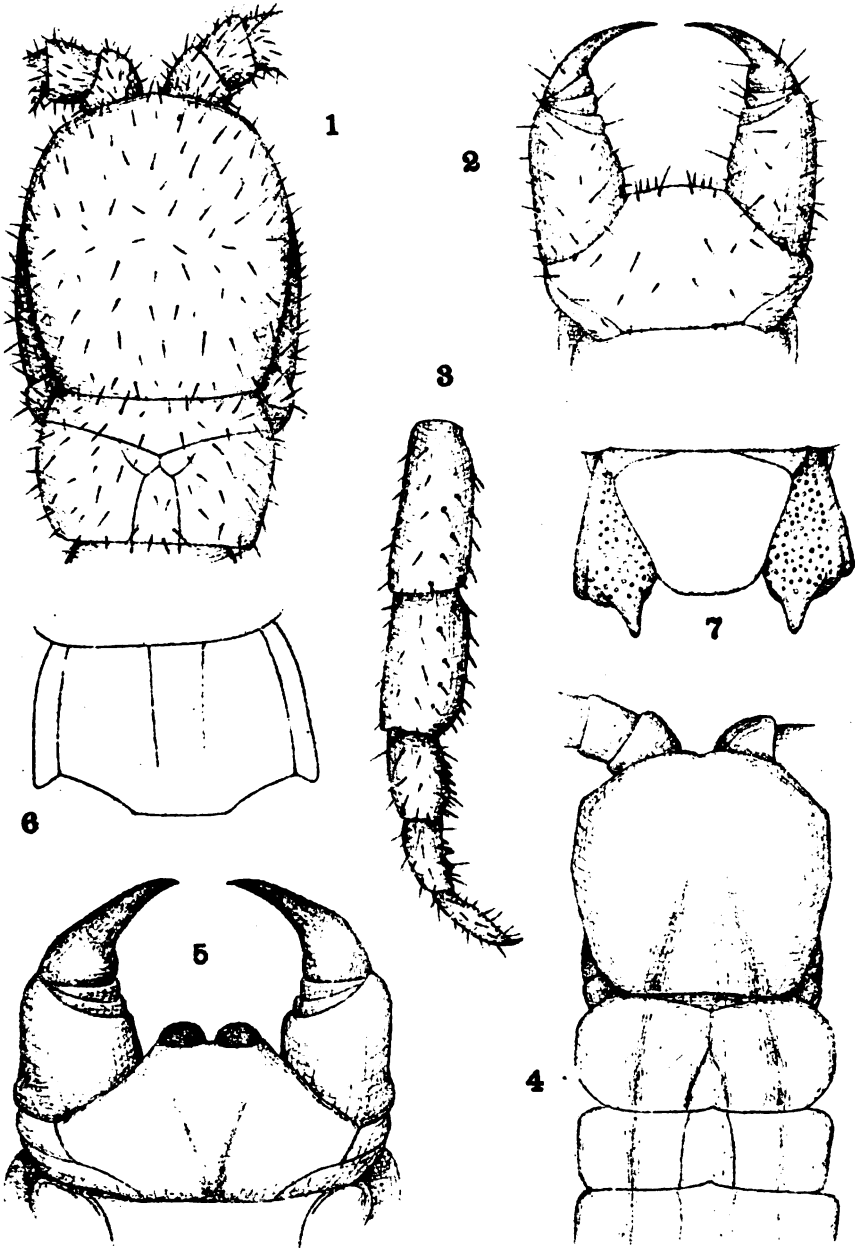


PLATE 2.

Newportia longitarsis sylvae Chamberlin.

1. Dorsal view of head and first dorsal plates.
2. Anal legs with distal articles of tarsus omitted.

Newportia paraensis, Chamberlin.

3. Anal leg.

Newportia amazonica Brölemann.

4. Lateral view of distal end of tarsus showing claw.

Newportia ernsti Pocock.

5. Lateral view of distal end of tarsus showing chitinous point or rudimentary claw occasionally present in some individuals.

Otostigmus tidius Chamberlin.

6. Prosternum and prehensors.
7. Last dorsal plate.
8. Last ventral plate.

Cupipes amazonae Chamberlin (cf. Plate 3).

9. Last ventral plate and coxopleurae.

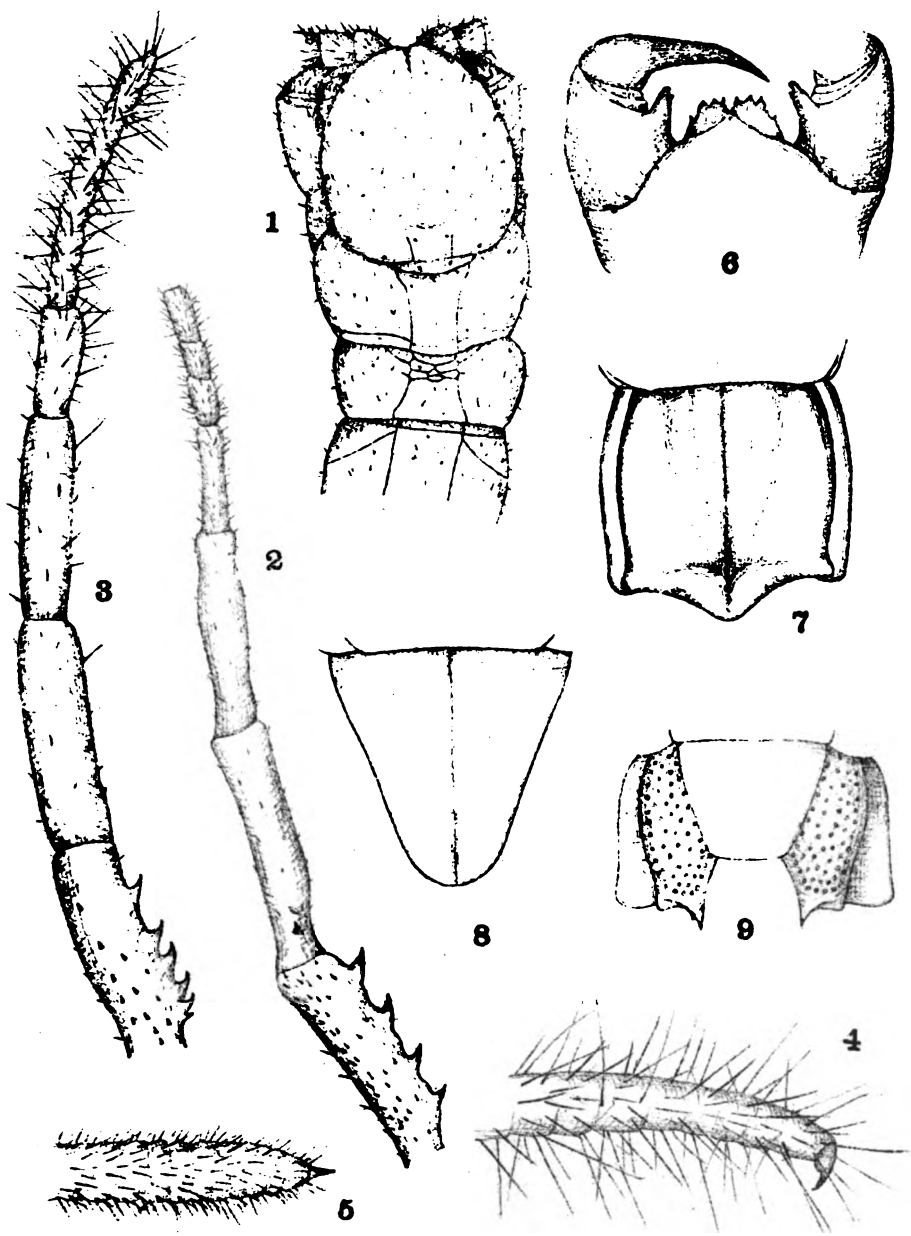


PLATE 3.

Otostigmus amasonae Chamberlin.

1. Last ventral plate.
2. Last dorsal plate.

Otostigmus suitus Chamberlin.

3. Last ventral plate.
4. Last dorsal plate.

Cupipes amasonae Chamberlin (cf. Plate 2).

5. Prosternum (in part and prehensors).
6. Distal portion of anal leg showing claw, etc.

Cupipes neglectus Chamberlin.

7. Prosternum (in part) and prehensors.
8. Last ventral plate and coxopleurae.

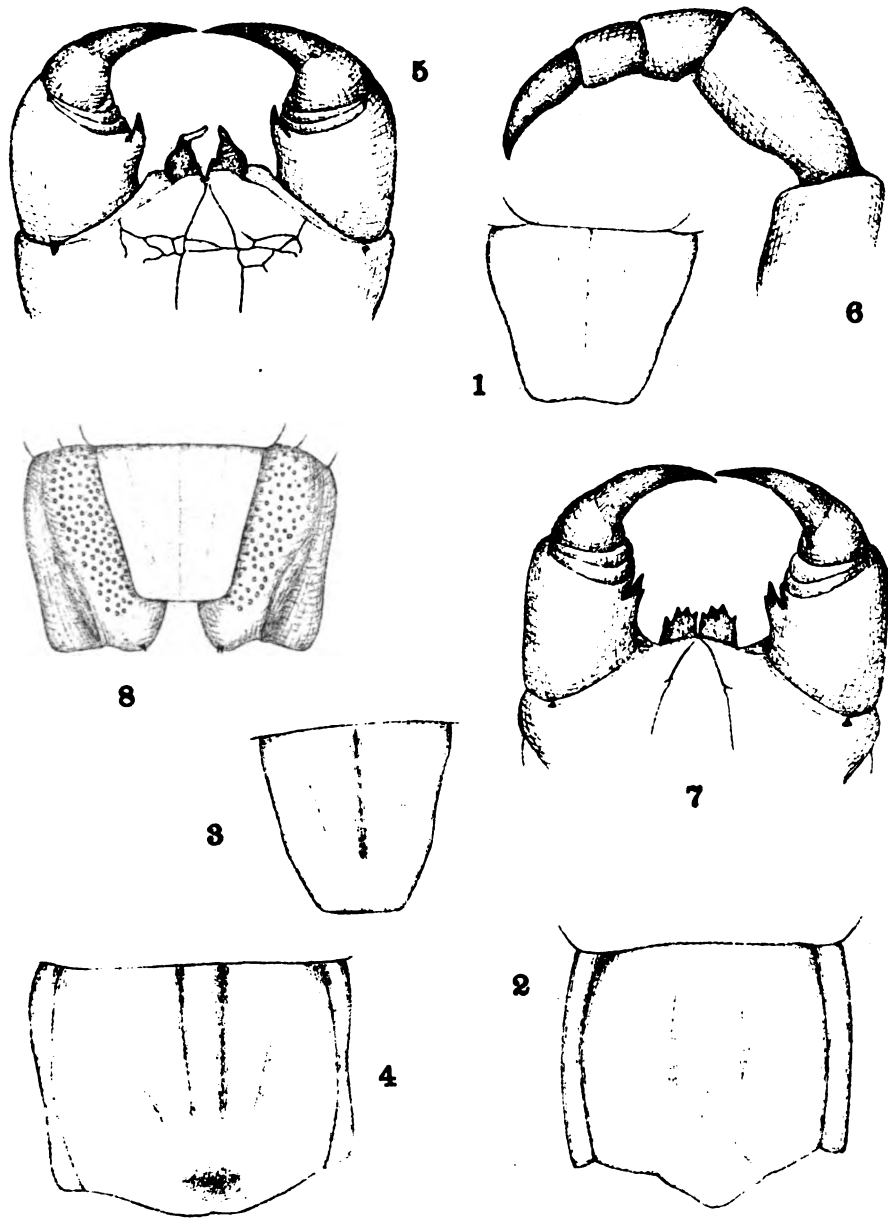


PLATE 4.

Schendylurus perditus Chamberlin.

1. Cephalic region, dorsal view.
2. Cephalic region, ventral view.
3. Caudal region, ventral view.
4. Antenna (on same scale of magnification as fig. 1, 2).

Schendylurus bakeri Chamberlin.

5. Cephalic region, dorsal view.
6. Cephalic region, ventral view.
7. Caudal region, ventral view.
8. Antenna (on same scale of magnification as fig. 5, 6).

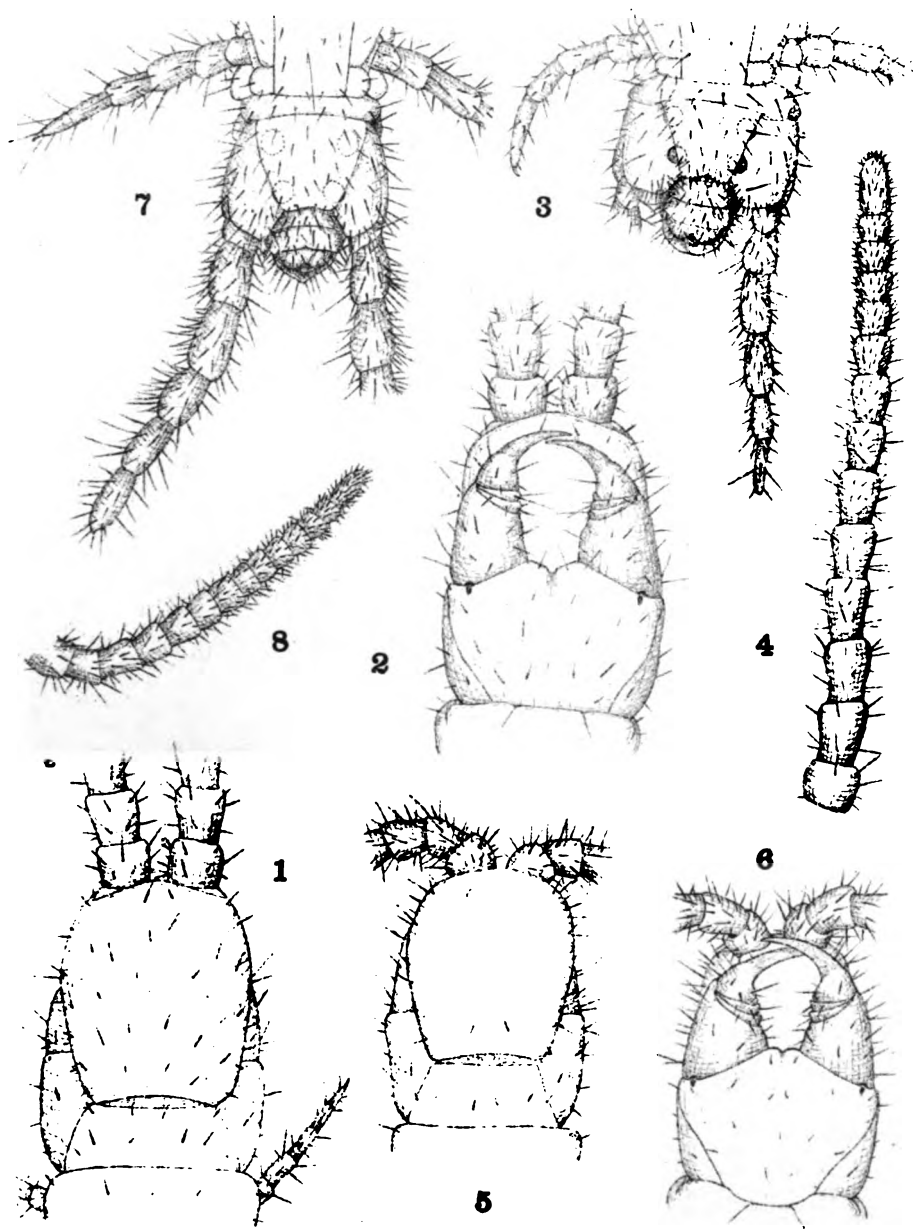


PLATE 5.

Adenoschendyla parahybae Chamberlin.

1. Cephalic region, dorsal view.
2. Cephalic region, ventral view.
3. Caudal regions, ventral view.

Orphnaeus branneri Chamberlin.

4. Cephalic region, dorsal view.
5. Cephalic region, ventral view.
6. Caudal region, ventral view.

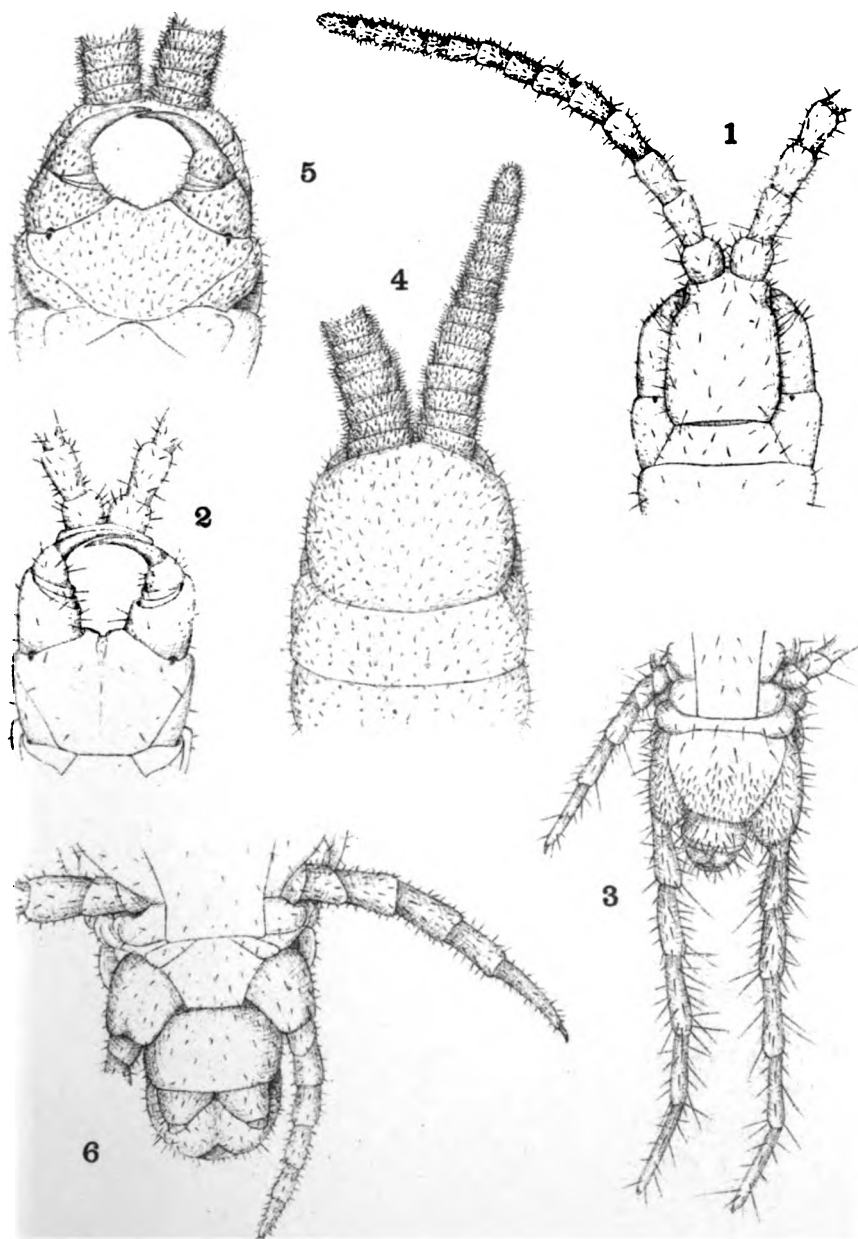
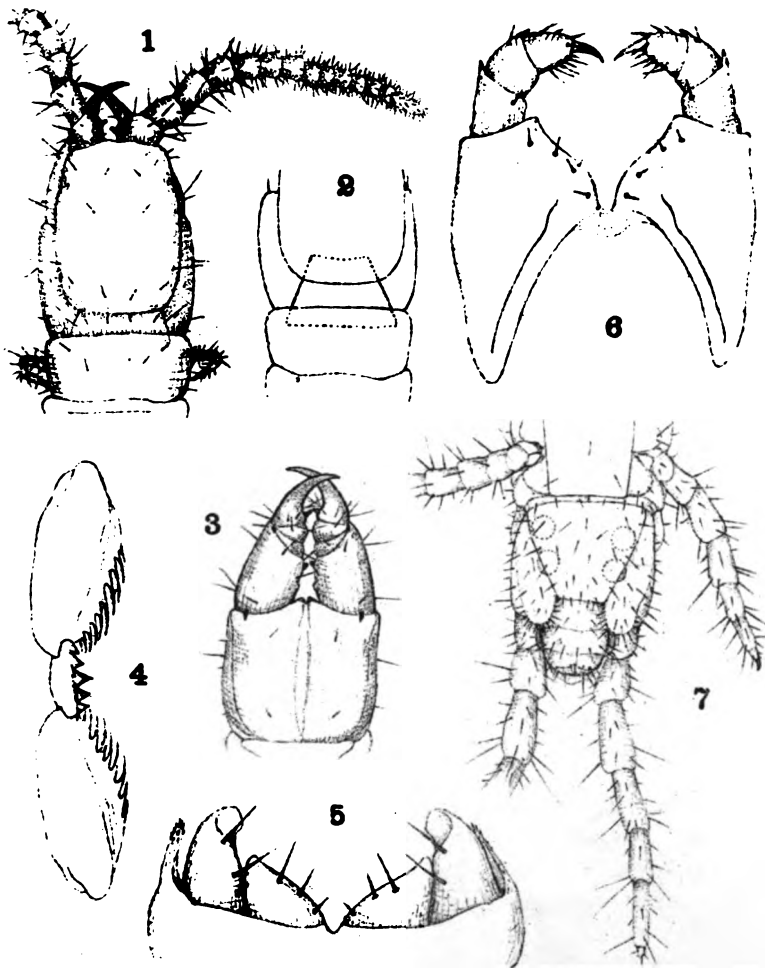


PLATE 6.

Schizonampa manni Chamberlin.

1. Cephalic region, dorsal view.
2. Outline showing relations of basal plate.
3. Prehensors, ventral view.
4. Labrum.
5. First pair of maxillae.
6. Second maxillae.
7. Caudal region, ventral view.



RESULTS OF A STATISTICAL STUDY OF VARIATION IN THE BLUE
SHELLS OF PECTEN NUCLEUS IRRADIANS FOUND AT
ATLANTIC CITY, N. J.

BY HAROLD S. COLTON.

Upon the beach of the Atlantic shore of New Jersey are found dead shells colored blue by clay particles infiltrated into the shell. These shells have obviously been buried in the mud or clay of the bottom, out of which they have been washed by the surf.

In collecting shells from along the beach at Atlantic City in 1904, the author fancied that the blue *Lunatia heros* differed in shape from the uncolored ones.

Not having sufficient material with which to base a conclusion, he returned in the Spring to Atlantic City to collect *Lunatia*. Although in January they were abundant, now not a single one could be found. At once it was seen that *Pecten* would be a much more favorable form on which to work, as it was abundant and the ribs of the shell would be easy to count, therefore a few hundred of white and blue ones were collected.

In the course of time the writer happened to mention to Dr. E. F. Phillips, the present chief of the Bureau of Apiculture at Washington, on what material he was working. Dr. Phillips became interested, and in 1905 a joint trip to the coast was made and over five hundred blue right valves of *Pecten* were collected near Ventnor.

The ribs of these were counted, applying the arbitrary rules of Davenport (1900). The number of ribs of each shell was counted independently and compared. When there was a difference of opinion the ribs were recounted, and no record was made until both agreed.

This study was not completed and therefore not published. While sufficient blue ones were at hand, not enough white ones (representing the form of *Pecten* now living) were collected for comparison. However, in looking over the results it seemed that it might be of interest to publish them as they

stand and let some one else take up the problem and bring it to a conclusion.

In some places along the Atlantic coast of New Jersey are exposed at low tide stumps of trees and also such turf as is now forming in the meadows behind the line of sand dunes. By this we can infer that the coast line is sinking. This being the case, the dunes are progressing inland, covering up the meadow turf and exposing it again in the ocean beach. Between the dunes and the mainland is oftentimes a bay or a channel. This bay or channel has a muddy bottom as a rule and there the *Pecten* lives. As the dune line progresses forward the meadow encroaches on the bay, burying the dead shells of the *Pecten* which are later exposed by the action of the surf and cast on the beach after the dune line has passed over them.

It would be interesting to test if such a theory were correct and note the direction of the evolution of the *Pecten* or other form during the time that they were buried.

An examination of well borings made in the beach might help throw light upon this point.

Experiments also might be made as to the rate of infiltration of the clay particles into the shell which may be rapid. The author made sections of the shells. These sections show that the clay has penetrated the shell to the center, the pearly layer alone excepted.

With the geologic evidence from well borings, with the experimental evidence from the rate of infiltration of the clay particles and the comparison of the variation of the blue forms with those now living, a conclusion might be drawn as to the direction of evolution in *Pecten*.

In the table which follows, N = the number of shells the rays of which were counted,— A = the mean,— σ the standard deviation from the mean,— C = the coefficient of variation,—and $P. E.$ the probable errors of the mean, the standard deviation, and the coefficient of variation. These have been computed by the ordinary methods. With them in lower case are placed similar counts made by Davenport (1900) on *Pecten irradians* from several other localities.

0.928

~~10.36~~

PECTEN irradians	Ray Frequency								N	A	P.E.A.	σ	P.E. σ	C	P.E.C.
	14	15	16	17	18	19	20	21							
BLUE PECTEN ATLANTIC CITY	6	72	187	185	44	5	1	.	500	16.416	$\pm .028$	.898	$\pm .020$	5.65	$\pm .12$
Cold Spring Harbor . .	2	15	108	515	308	90	7	1	1046	17.35	$\pm .018$	.876	$\pm .013$	5.049	$\pm .074$
Cutchogue . .	.	33	95	127	22	4	.	.	281	16.53	$\pm .034$	.852	$\pm .024$	5.15	$\pm .15$
Fire and Oak Islands . .	1	6	15	24	4	.	.	.	50	16.48	$\pm .084$	.877	$\pm .060$		

These comparisons show that the average number of ribs of the blue Pecten at Atlantic City is less than the same species at Cold Spring Harbor but about the same as at Fire Island and Cutchogue. That they are more variable than any of these that Davenport reported is also shown by the statistics. To determine the significance of these differences will require much more work.

The author wishes to thank Dr. E. F. Phillips for his share in the work, and also for first introducing him to modern statistical methods.

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IX.

PHYSIOLOGICAL STUDIES ON CERTAIN PROTOZOAN
PARASITES OF *DIADEMA SETOSUM*.

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PHYSIOLOGICAL STUDIES ON CERTAIN PROTOZOAN PARASITES OF DIADEMA SETOSUM.

BY MERCEL H. JACOBS.

INTRODUCTION.

It has been shown by the author and others that different species of protozoa have certain physiological characteristics, often almost as striking as their morphological ones, and which are probably of considerable significance in the interpretation of their habits of life and their relation to their environment. The study of such characters in the past has been greatly neglected on account of the general belief of biologists that they are exceedingly inconstant and subject to modification by external factors and therefore of little value in an understanding of the fundamental nature of the organisms possessing them. Previous attempts to detect characteristic physiological differences between different forms have for the most part been confined to organisms whose general habits of life are so dissimilar as to leave room for the objection that the differences observed may have been due merely to the effect on the animals of the different environments to which they have been accustomed and not to any innate peculiarities of the organisms themselves.

It occurred to the author as desirable to test, if possible, a series of forms which naturally live under essentially the same environmental conditions, and which may be assumed to have done so for many past generations, in order to see whether they show greater likenesses than a number of forms selected at random, or whether each has preserved its individuality in spite of the similarity of its environment. In the case of a number of parasitic protozoa living together in the same organ of the same host, we have a favorable opportunity for such a study. Even here we can not assume that each form has exactly the same environment, but, there can be no question that this environment is far more similar than that of a number of selected free-living forms. Furthermore, in order to become parasites of the same host the nature of the forms in question must have been more or less changed. In the case of intestinal parasites, for example, it would be necessary for them to acquire a resistance to the digestive juices of the host and to become accustomed to a more or less anaerobic habit of life, etc. Such changes would necessarily be in the same direction for all of them. If, therefore, a number of parasitic forms, which have had to meet and adapt

themselves to the same new conditions and live under them together for thousands of generations, still show as characteristic physiological differences as free-living forms, it is good evidence of the fundamental nature of these differences. Is such the case? The present paper is a preliminary consideration of this question. The work on which it is based was performed at the Tortugas Laboratory of the Carnegie Institution of Washington during the summer of 1910.

MATERIAL.

The material used in these experiments consisted of four species of ciliate protozoa obtained from the alimentary tract of the large black sea-urchin, *Diadema setosum*, which can be procured in practically unlimited quantities from the coral reefs about the Tortugas. This form is particularly favorable as a source of material, not only on account of its abundance but on account of the high percentage of infection shown by it with respect to the protozoa in question. Out of over 100 adult *Diademas* examined during the progress of the work, not a single one failed to show one or more kinds of protozoa in the intestine, which often fairly swarmed with them. Doubtless the large size of the intestine, its pouch-like recesses in which stagnation of the food can occur, and the character of the food itself are factors favoring the presence of protozoan parasites or, perhaps more correctly, protozoan commensals, since none of the forms in question seem to be injurious to the host or to live at its expense, except in so far as they use up a small portion of its practically unlimited supply of food.

The forms studied were of four kinds. Since apparently none of them have been previously described or named, they are for convenience designated in the account that follows by the letters, *A*, *B*, *C*, and *D*. Of the four, *B* is the most regular in its occurrence, having been found in 100 per cent of the adult *Diademas* examined. It is a large, heterotrichous form allied to *Metopus*, with a hook-like anterior end and showing considerable individual variation in appearance.

The form next in abundance is *C*, which is found in about 75 per cent of the cases. It is a medium-sized, holotrichous form, elongated in shape, with the anterior end of the body transparent and longitudinally striated and the remainder filled with refractive spherules. A nucleus is visible about the middle of the body and no mouth opening can be detected.

The next form in importance is the one designated as *D*, which is found in 40 per cent of adult *Diademas*. It is flat and leaf-like in form, with the body slightly concave on the ventral and convex on the dorsal surface. The one margin of the body is almost straight and the other curved, giving the animal an asymmetrical shape. At the posterior end is a spur-like projection. Evidently this form is allied to the holotrichous genus *Dysteria*.

The form which occurs in the smallest number of cases (33 per cent) is *A*. When present at all, however, the number of individuals is generally very large. In size it is rather small and in shape elliptical and flattened.

Very little could be made out of its structure, owing to its small size, rapid movements, and its peculiar tendency to disintegrate with almost explosive suddenness when kept under observation on a microscopic slide. In addition to these four characteristic forms, several other ciliates were observed more rarely, and on one occasion an *amæba*. They were not found frequently enough, however, to be considered in the experimental part of the work. So far as the present observations go, the important group of flagellates appears not to be represented by parasitic forms in *Diadema*.

The parasites of *Diadema* seem to occur exclusively in the alimentary tract, none being found in the body-fluid so long as the intestine is uninjured. In the alimentary tract itself the number is not the same in the different regions, some parts being more favorable than others. The greatest numbers are found in the distended pouches of the second, or upper, coil of the intestine. In the lower coil they seem to be almost or entirely absent, even when present in great abundance elsewhere. It is interesting to note in this connection that free-living forms (e. g., *Foraminifera*, etc.), which are taken in with the food, are invariably dead before the second coil is reached. The rectal portion of the intestine also shows very few protozoa, form *D* being the one most frequently encountered there, though *B* and *C* may occur rarely and in small numbers. On several occasions cysts, possibly of *D*, were found in this region. It seems quite probable that cysts thus formed may pass out and eventually infect other *Diademas*. It does not always happen that the different protozoa present show the same distribution, thus, *A*, for example, may be quite absent in some of the pouches and present in others, while *B*, *C*, and *D* show similar irregularities. It is usual to find several of the forms together, but there are no constant combinations; any form may occur associated with any of the others.

The above remarks concerning the abundance and distribution of the parasites apply only to adult *Diademas*. As might be expected from our knowledge of other animals, young individuals contain either no parasites at all or at most but a few. The youngest ones examined (0.5 inch in diameter) contained nothing. Somewhat older ones (1.5 inches in diameter) contained sometimes nothing at all, sometimes large numbers of a form not found in any of the adults examined, and in a few cases small numbers of *C* and *D*. It is rather striking that form *B*, which appears to be present in all adults, should be so constantly lacking in younger individuals. Whether this is because conditions for its existence are unsuitable or simply because no opportunity for infection has occurred could not be determined.

A search for the parasites in question was also made in sea-water and in other animals, related and unrelated to *Diadema*. The only situations in which any of them were found was in the alimentary tract of other sea-urchins, where they may occur in varying numbers. *B*, for example, was found rarely in *Toxopneustes* and *Echinometra*, and *C* on a number of occasions in *Toxopneustes*, while *D* seems to be present in every adult *Toxopneustes*, and rarely in *Echinometra*. *A* was never found outside of

Diadema. A number of specimens of *Hipponoë* were examined for protozoan parasites without results.

METHODS.

In comparing the physiological nature of the forms in question certain characters were chosen which could be expressed quantitatively and the animals examined with respect to them. Those selected all had to do with the length of time the organisms could maintain their normal activities under various conditions differing from those to which they were accustomed. The method adopted was to place the animals under the new conditions and note the time that elapsed until all visible movements ceased. In the case of ciliates this time corresponds very closely with that at which irreparable injury, resulting in death, has been done to the cell, and therefore furnishes a more or less accurate criterion of the general vitality of the organisms.

In the present experiments the number of individuals studied was always large and the range of variation within the species small, and therefore it was found possible to choose, as the point for comparison, the time at which all the individuals of the given species had been rendered motionless. This point was easy to determine and the general agreement in the results obtained on successive days by the use of the above method showed it to be sufficiently accurate in a case, such as the present one, where it was desired to express the relative and not the absolute resistance of the four forms in question. Most of the experiments were repeated a number of times with material obtained from different sources, so as to eliminate accidental errors. The characters, in respect to which the different forms were compared, were as follows: (1) Ability to live outside the body of the host; (2) ability to live in the body-fluid of other related animals; (3) length of life of the parasites after the death of the host; (4) resistance to CO_2 ; (5) resistance to H_2S ; (6) resistance to the decomposing proteid substances; (7) resistance to H_2SO_4 ; (8) resistance to KOH .

EXPERIMENTS.

ABILITY OF THE PARASITES TO LIVE OUTSIDE THE BODY OF THEIR HOST.

It is well known that protozoan parasites show a varying ability to live apart from their host. In general, the less specialized the parasite and the looser the relation between it and its host, the longer it may be expected to live under conditions other than the natural ones. The forms in question are not highly specialized and, as might be supposed, are for the most part able to live for a considerable time outside the intestine of *Diadema*, though so far as these experiments go, a continued existence of this sort is in no case possible. The point of particular importance, however, from the standpoint of the present investigation, is that the four forms studied show marked differences in behavior when removed from the body of their host. If a number of food pellets from the intestine of a *Diadema* be shaken up with a little fresh sea-water, none of the parasites obtained in this way is at first

visibly injured. If, however, a few cubic centimeters of the mixture be placed in a covered glass dish which prevents evaporation but which, being only partially filled, does not prevent contact of the liquid with the air, it will be seen that all of the animals present will die after a length of time which is fairly constant for each species. The first one to disappear apparently always is *A*, which is no longer present after 2 to 3 hours.

It may be mentioned that no way of keeping *A* longer than this in artificial cultures was found except to place a relatively large quantity of the intestinal contents in a test-tube with the addition of only sufficient water to keep it moist, in which case the animals retained their activity somewhat longer. *D* is next to *A* in respect to its behavior in sea-water culture, and usually is dead at the end of 24 hours, though a few individuals may survive several hours longer. *C* does not differ very markedly from *D*, but is somewhat more resistant, living on an average 30 hours or a little over. *B* is by far the most resistant form, often surviving and continuing active for 2 or 3 days. The relative ability of the four forms to live outside the body of their host may therefore roughly be expressed by the ratio 1 : 24 : 12 : 10.

To determine to what extent the injurious effects of removing the parasite from their host were due to the action on them of the oxygen of the air, a parallel series of experiments was tried in which oxygen, so far as possible, was excluded. This was done by shaking up the food pellets from the intestine with sea-water from which the air had been removed by boiling (the concentration of salts being kept constant by the addition of distilled water) and immediately placing the liquid in dishes which were completely filled, covered, and sealed with vaseline to exclude the air. Such treatment was found not materially to alter the length of life of any of the forms except *B*, which in one such experiment lived for 7 days, the longest time for which it was found possible to keep any of the forms alive by any of the methods employed. *A*, *C*, and *D* died in about the usual times, the life of *A*, if anything, being slightly shortened and that of *C* slightly lengthened. In no case except that of *C*, however, were the differences significant.

ABILITY OF THE PARASITES TO LIVE IN THE BODY-FLUID OF TOXOPNEUSTES.

Early in the course of these experiments it was observed that the forms in question live in cultures made with the body-fluid of *Diadema* about the same length of time that they do in sea-water. This is not surprising, since the osmotic properties of the body-fluids of sea-urchins and sea-water are about the same. A point of some interest seemed to be whether the body-fluid of a nearly related genus of sea-urchins would be as favorable as that of the host or as sea-water, or whether there might be present in it in addition to the salts of sea-water certain other substances of more specific nature which would have a toxic effect. To determine this point, therefore, experiments were made, but unfortunately they were not very extensive. One such experiment may be mentioned. *A*, *B*, and *C* obtained from *Diadema* were placed in a small quantity of the body-fluid of *Toxopneustes*.

A was found to be dead at the end of 1.5 hours, *B* in 3 hours, and *C* was living after 15 hours, but dead in 24. In a control culture in which the body-fluid of the original host was used in place of that of *Toxopneustes*, *A* lived about 2 hours, *B* was still active at the end of 24, and *C* died somewhere between 15 and 24. It will be seen, therefore, that *A* and *C* are less affected by the change than is *B*, which normally lives 10 to 20 times as long in the body-fluid of its own host as in that of *Toxopneustes*. Unfortunately *D* was not available at the time when this experiment was performed. The converse of the above experiment, namely, the subjecting of parasites obtained from *Toxopneustes* to the body-fluid of *Diadema*, was also tried with similar results. In this case only *D* was available, it being the only one of the four forms found in quantity in *Toxopneustes*. In the control culture in which the body-fluid of their own host was used the animals were normal after 15 hours; in the body-fluid of *Diadema* they were dead in 2 hours.

The injurious effects of transferring the parasites to the body-fluid of an animal other than their host are almost certainly not due to physical (*e. g.*, osmotic) differences between the body-fluids of the two animals, since they may be transferred with impunity from either host to sea-water, the body-fluid of sea-urchins having approximately the same osmotic properties as the latter. Furthermore, in a number of experiments in which the concentration of the liquid surrounding the parasites was purposely suddenly altered, the forms in question were shown to be quite resistant to changes of this sort. It may be assumed, therefore, that chemical rather than physical differences in the two body-fluids are responsible for the death of the animals. It is rather interesting that different races of the same species are found adapted to different hosts and apparently can not be suddenly transferred from one to the other. Where adaptive characters such as these are concerned, we can look for a considerable amount of variation within the species. But in the case of non-adaptive characters, such as some of those mentioned in the succeeding experiments (resistance to H_2S , H_2SO_4 , etc.), it was found that the properties of form *D*, for example, were practically the same whether it was obtained from *Diadema* or *Toxopneustes*, and consequently that such characters are fairly constant for the species.

LENGTH OF LIFE OF THE PARASITES AFTER THE DEATH OF THE HOST.

If a dead *Diadema*, either opened or unopened, be covered with sea-water and allowed to stand in a warm room, the number of parasites present soon begins to diminish and after a variable time all disappear. Sometimes in as short a time as 12 hours none are to be found, though in other cases they may be active for 24 hours or more. In no instance, however, do they seem to persist more than 30 hours at the room temperature of the Tortugas laboratory (85° to 95° F.). They usually disappear in the order, *D*, *A*, *C*, *B*. In one series of observations *D* was dead in 12 to 15 hours, *A* in a little less than 20, while *C* and *B* survived somewhat over 24. While the absolute

time of disappearance differs in different experiments the relative order for the four forms seems to be quite constant.

It will be seen, therefore, that in respect to their ability to live in the body of the host after the latter's death, just as in many other respects, they show certain characteristic physiological differences. The relative length of life of the four forms is roughly 1.5 : 2 : 2 : 1. Just what factors cause the death of the parasites under these conditions is not certain, but probably the products of decomposition present play an important part. As will be shown later, some of these substances are markedly toxic, though each of the four forms is affected differently by them and the immediate cause of death may perhaps be different in the different cases.

EFFECTS OF CO₂ ON THE PARASITES.

Carbon dioxide certainly occurs normally in the intestine of *Diadema* and therefore small quantities of it can not be markedly injurious to any of the four forms in question. With large quantities the case is different, all being killed in a fairly short time, though the four forms are by no means alike in their resistance. If a drop of fluid containing them be exposed to a continuous stream of the gas in a gas-chamber and kept under constant observation, it will be seen that *D*, the least resistant form, is rendered motionless in 3 or 4 minutes; *A* comes next, the time required for it being 5 to 6 minutes; *C* remains active for about 15 minutes, and *B* for from 45 to 60 minutes. The ratio of resistance is therefore roughly 1.5 : 15 : 4 : 1; *B* being the most resistant form and *D* the least. The same order of resistance was found to hold if instead of subjecting the animals to a stream of gas they were placed in a relatively large quantity of sea-water charged with CO₂ by means of a "sparklet" bottle.

EFFECT OF H₂S ON THE PARASITES.

Hydrogen sulphide begins to appear in the intestine of *Diadema* in considerable quantities soon after death. The vapors given off on gently warming a little of the intestinal contents taken 12 hours after death darken lead acetate paper very perceptibly, and after 24 hours the reaction may often be obtained without the application of heat at all. As this substance might be suspected of playing a part in causing the disappearance of the parasites from the intestine of the host after death, it was of interest to examine its effects on the four forms in question. As might be expected, it proved to be markedly toxic to all, but the different forms resisted it in pronouncedly different manner, and, furthermore, the order of resistance is different from that shown to CO₂. *C*, for example, which is next to the most resistant form to CO₂, is killed first when a current of the gas is passed through the gas-chamber containing the animals, the time required being only 1 to 2 minutes; *D* comes next, with a period of about 3 minutes; *A* lives 3 to 4 minutes, and *B* 10 to 15. The ratio of the resistance of the four forms is therefore approximately 2.5 : 8 : 1 : 2.

EFFECT OF DECOMPOSITION PRODUCTS ON THE PARASITES.

In an attempt to throw light on the cause of the disappearance of the parasites after the death of the host, the effect on them of the direct products of the decomposition of the tissues of *Diadema* was tried. The method used was to allow fragments of *Diadema* tissues to decay in the water for 3 days at room temperature; 5 drops of the resulting infusion were then added to 10 drops of the fluid containing the animals. The results were most surprising. *B*, which had hitherto proved the most resistant form in most of the experiments, was killed almost instantly—certainly in less than 5 seconds; *A* lived from 10 to 60 seconds; *D* 20 to 25 minutes; and *C* as long as 2 hours. *C*, therefore, which is less resistant than *B* to most conditions, is in this case many more times resistant. The ratio for the four forms is roughly 7 : 1 : 1,500 : 250. These figures seemed so remarkable that the experiment was repeated several times, but always with essentially the same results.

THE EFFECT OF H_2SO_4 ON THE PARASITES.

A limited number of experiments were tried with this substance. It was necessary first to determine the proper concentration, since if the solution is too strong death occurs so quickly that its time can not be accurately observed, and if too weak it occurs so late that other factors have time to complicate the results. A series of preliminary experiments showed that a favorable proportion is 1 drop of N/10 H_2SO_4 to 5 drops of the fluid containing the organisms. Of course, care had to be taken that the acid was immediately mixed thoroughly with the culture medium, otherwise the organisms were not all subjected to exactly the same conditions. Treated in this way, the four forms showed striking differences: *A* had a very low resistance, being killed in about 5 seconds; *D* was killed in less than 30 seconds, but the exact time (somewhere between this and 5 seconds) was not noted; *C* died in about 30 seconds; while *B* survived 1.25 hours or 900 times as long as *A*. This is seen to be a surprisingly high resistance to acid when it is remembered that *Paramecium* and many other fresh-water forms are killed almost instantly by exposure to N/500 H_2SO_4 . Summarizing the results of the observations on the resistance of the four forms to H_2SO_4 we have the ratio, 1 : 900 : 6 : < 6.

EFFECT OF KOH ON THE PARASITES.

This substance used in the same concentration as H_2SO_4 , as might be expected from results obtained with other animals, is on the whole much less injurious. *A* (the least resistant form) was found to be killed in about 2 minutes, *D* in somewhat less than 45 minutes, *B* in about 1 hour, and *C* in 3 to 4 hours. It will be noted that while *A*, *C*, and *D* are much more resistant to KOH than to H_2SO_4 , *B* is actually less resistant. The ratio for the four forms is roughly 1 : 30 : 100 : 20.

CONCLUSIONS.

The general results of the experiments performed is to show surprising differences in the resistance of the parasites of *Diadema* to various unfavorable conditions. In some cases the most resistant form may live several hundred times as long as the least resistant one. *B* is 900 times as resistant to H_2SO_4 as *A*, and the difference between *B* and *C* with respect to products of decomposition is still more striking. Furthermore, a form which is strongly resistant to one condition may be only feebly so to another, and vice versa. For example, *B* is 8 to 10 times as resistant to H_2S as *C*, but 1,500 times less resistant to certain of the products of decomposition of *Diadema* tissues. *C* is more resistant to CO_2 than *A* or *D*, but less resistant to H_2S . *A*, *C*, and *D* are 24 to 450 times more resistant to KOH than to H_2SO_4 , while *B* is somewhat less resistant. Other similar facts could be mentioned.

Comparing all of the results obtained, it is therefore seen that the similar habit of life of the four forms in question has not brought about physiological similarity except in certain adaptive characters which are a *sine qua non* for continued existence in the same host (*e. g.*, ability to resist the digestive juices of the latter, etc.). In other respects they are just as different as almost any four free-living forms that might be selected and so far as the evidence of these experiments goes it seems to show that the physiological characters of an organism are not merely the result of its environment, but may be as fundamental and characteristic as its morphological ones.

ANTAGONISM BETWEEN SALTS AND ANÆSTHETICS. —
III. FURTHER OBSERVATIONS SHOWING PARALLEL
DECREASE IN THE STIMULATING, PERMEABILITY-
INCREASING, AND TOXIC ACTIONS OF SALT SOLU-
TIONS IN THE PRESENCE OF ANÆSTHETICS.

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I. INTRODUCTORY.

THE present paper forms a continuation of several previous contributions to the same general subject. In the experiments described in these papers the chief aim has been to obtain direct evidence of the part played by the semi-permeable limiting membranes of cells in physiological processes, especially in stimulation and in certain forms of toxic and antitoxic action. It has seemed probable that if changes in the permeability of the plasma membranes of the irritable elements form an essential part of the general process of stimulation,¹ as there is good reason to believe, any artificially produced modifications of irritability would be found associated with corresponding modifications in the properties of the membranes. Thus in narcosis or other conditions of hypo-irritability we should expect that the permeability of the membranes would undergo alteration with less readiness than normally, and that the cells would exhibit an increased resistance to those forms of injurious or toxic action which consist in the production of an abnormally increased surface permeability. Experiments on *Arenicola* larvæ and echinoderm eggs have shown, in fact, that the rapid permeability-increasing action of pure solutions of sodium chloride and other neutral salts is checked or prevented in the presence of anæsthetics, and that simultaneously

¹ Cf. this Journal, 1911, xxviii, p. 197; 1909, xxiv, p. 14.

the characteristic stimulating and toxic effects of these solutions undergo a marked decrease.²

Narcosis, as a physiological phenomenon, is interesting chiefly because of the uniformity both of its manifestations and of the conditions under which it is produced. Activities of the most various kinds (cell division, stimulation and conduction, contractile processes, secretion, growth) are diminished or abolished, reversibly, in the presence of certain substances. Of these the most universal in their action are the numerous largely water-insoluble organic compounds possessing solvent action on fats and lipoids. Since protoplasm appears always to contain lipoids, a selective action on these substances seems thus to be indicated as the basis of anæsthesia. Overton and Meyer found that a parallelism between the water-lipoid partition coefficients of the lipoid-solvent anæsthetics and their narcotic action did in fact exist, and they accordingly referred narcosis to an alteration of the lipoids. The question as to why lipoid alteration should decrease or abolish irritability remained, however, unsettled. Narcotic action is also exerted by various substances — salts, non-electrolytes, acids — which have no specific relation to lipoids, and also by the electric current under certain conditions (anelectrotonus). Apparently, therefore, specific alteration of the lipoids is not the essential basis of narcosis, although such a change may induce this state. The indications point toward some more general kind of modification in the irritable elements; this modification may be caused by, but is not necessarily connected with, a change in the condition of the lipoids.

In anæsthesia we have to do with a change in irritability. The problem of the physiological nature of anæsthesia is thus inseparable from the more general problem of the nature of the stimulation process. The influence of anæsthetics on irritability must therefore be studied from the general point of view of this latter problem. It should be noted that these substances do not invariably *lower* irritability, but do so only in certain strengths of solution; below these concentrations they frequently increase the responsiveness or degree of automatic activity of the tissue. Thus traces of ether and other anæsthetics increase the rate of the heart beat, of growth and cell

² Cf. this Journal, 1912, xxix, p. 372; xxx, p. 1.

division, of ciliary movement, of amoeboid movement.³ In higher concentrations these activities are checked or brought to a rest, reversibly. In still higher concentrations cytolytic action sets in. There are thus ranges of concentration corresponding to quite different physiological effects. The same phenomenon is seen with certain salts — for example, those of calcium. Irritability may thus be modified in either direction; an irritable tissue may be sensitized or desensitized by appropriate concentrations of a given lipid solvent or other substance. The terms “sensitization” and “desensitization” may have reference not only to changes in the responsiveness of tissues or organisms to stimulation, but also to changes of susceptibility to particular poisons. This is probably no merely verbal coincidence. A parallelism between the two effects, irritability-increasing and susceptibility-increasing, exists in certain cases,⁴ and there is evidence that the essential physico-chemical basis of both kinds of effects is the same, and consists in an alteration in the normal properties of the plasma membranes, of such a kind that the increase of permeability essential to both the stimulating and the toxic action of the poisonous substance (*e. g.*, saponin) is facilitated or promoted. Conversely, anæsthetics desensitize the tissues of *Arenicola* larvæ to both the stimulating and the toxic action of pure isotonic NaCl solutions.

There now exists ample evidence that the initial or critical event in stimulation consists in a change in the condition of the surface film or plasma membrane of the irritable element. The passage of an electrical current through the tissue necessarily causes a change in the electrical polarization of the semi-permeable membranes, and it is this change (as shown by Nernst and his successors) which forms the critical or primary process upon which follow the chemical, mechanical, or electrical manifestations (or response) characteristic of the tissue. It is well known, however, that mechanical impact, heat, and various chemical substances may call forth in a nerve or muscle

³ Instances of these effects are cited in the first paper of this series: this Journal, 1912, xxix, p. 374.

⁴ *E. g.*, the toxic action as well as the stimulating action of saponin and other poisons on frogs' voluntary muscle is greatly increased if the muscle is previously “sensitized” by immersion for a few minutes in a pure isotonic solution of a sodium salt. Cf. this Journal, 1911, xxviii, p. 214.

the same response as the electrical current; the essential or determinative change in stimulation must therefore be one which is produced in common by all of these agencies. Now the facts of electrical stimulation, significant as they are, do little more than *localize* this primary or critical action at the semi-permeable membranes of the tissue; the precise nature of the change there produced has to be determined by other means; and it remains to inquire what kind of surface change is adequate to account for the most characteristic and constant features of stimulation, as well as for the possibility of its being caused by so many different agencies. In brief, the evidence indicates that the properties of the limiting membranes undergo temporary alteration during stimulation; the plasma membranes, semi-permeable during rest, appear during stimulation to become more permeable than before.⁵ Stimulation, in other words, is associated with an increase in the general permeability of the limiting membranes. This consideration explains why agencies other than the electric current stimulate; they act by directly altering the permeability of the membrane. Such a change would account for the electrical variation which so constantly accompanies stimulation, and also for such characteristic effects as the refractory period, as well as for the osmotic effects which appear in some cells (gland cells, pigment cells, egg cells after fertilization) and indeed in certain cases, as in motile plant tissues, form the essential condition of the response.

If this hypothesis is well based, the condition of the membrane at any time must affect the readiness with which its permeability undergoes change, and hence also the responsiveness of the tissue to stimulation. This consideration explains the characteristic effectiveness of lipid solvents in altering irritability. The general properties of plasma membranes indicate that lipoids enter largely into their composition. Both animal and plant cells are as a rule readily permeable to lipid solvents and lipid-soluble substances, as Overton was the first to show; and these substances in higher concentrations have a specific action in increasing the permeability of the membranes. If the characteristic properties of the plasma membranes depend largely on the condition of the component lipoids, it is to be expected that lipid-modifying substances will influence the readiness with which

⁵ For a general review and theoretical discussion of this evidence *cf.* my paper in this Journal, 1911, *loc. cit.*, p. 197.

the permeability of the membrane is altered by various agencies, and also — if an increase of permeability is essential to stimulation — that they will at the same time modify the readiness with which stimulation is effected. These two effects ought theoretically to show a certain parallelism. In other words, any anæsthetic which decreases the susceptibility of a tissue to stimulation ought to decrease at the same time its general susceptibility to permeability-increasing or cytolytic action. The experiments about to be described show that this is in fact the case, so far as regards the action of neutral salt solutions on the tissues of *Arenicola* larvæ.

In an investigation like the present, which aims at determining the relation between permeability alteration as influenced by anæsthetics and stimulation as influenced by the same substances, it is indispensable to obtain as object of experiment an organism which will show simultaneously and in a clear and unmistakable manner both the changes of permeability and the stimulating effects caused under given conditions, *e. g.*, by a certain solution. Higher organisms are obviously unsuitable for this purpose; in these the differentiation is too detailed and the physiological conditions are too complex. Such a satisfactory test organism — or physiological indicator — is to be sought only among the lower forms of life, and I have found the free-swimming larvæ of the common shore annelid *Arenicola* remarkably well adapted to this purpose. These organisms have the advantages of being readily obtainable in large quantities, of showing great constancy in their reactions, and of possessing a sufficient but not too detailed differentiation of cells and tissues. The musculature is well developed and responds normally to electrical and other forms of stimulation — *e. g.*, the law of polar stimulation is shown typically; external locomotor cilia are present, and a yellow pigment (appearing brown when the larvæ are massed) is abundant in the body cells, and passes readily into the water when the permeability is increased by cytolytic or other agencies; this substance thus serves as a convenient index of the degree of permeability increase.

I have elsewhere described the action of pure isotonic NaCl solution on *Arenicola* larvæ.⁶ The visible effects which immediately follow the addition of the solution and indicate most clearly the essential nature of its primary action may be described as of three chief kinds,

⁶ Cf. this Journal, 1909, xxiv, p. 23.

relating respectively to the musculature, the pigment-containing cells, and the cilia. These effects are briefly as follows: (1) There is a strong and persistent muscular contraction, causing the larvæ to shorten to about half their normal length; they remain thus contracted for twenty or thirty seconds and relax slowly, regaining their original length within about a minute after the first contact of the solution; all muscular movements then cease, and during their further stay in the solution the larvæ remain quite motionless. (2) The yellow pigment contained in the body cells diffuses rapidly into the solution, and if sufficient larvæ are present imparts to this a distinct straw-yellow tinge. (3) The locomotor cilia cease movement almost immediately, and shortly afterwards many undergo a visible disintegration or breakdown into minute droplets or granules; usually there is some variation in resistance, and a few cilia may continue feeble movement for a minute or two and occasionally longer; but typically all trace of activity ceases within five minutes or less, and there is no revival when the larvæ are returned to sea water. A stimulating, a permeability-increasing, and a direct disintegrative action can thus be distinguished. In addition to these visible external effects there is a general toxic action affecting the entire organism. Larvæ returned to sea water after a few minutes (less than five) in the pure NaCl solution are found to have permanently lost their normal heliotropism; muscular contractions return after an interval, but slowly and imperfectly; there is no revival of ciliary movement, and the larvæ die within a few hours. If the return to sea water is delayed for several hours, there may be no signs of revival.

The above effects indicate that the salt solution acts primarily on the solid colloidal structures, especially the surface structures, of the tissues. Apparently the colloids undergo immediate changes of aggregation state, which lead in the case of the cilia to a structural breakdown and in the case of the tissue cells to an abnormal increase in the permeability of the limiting membranes. The latter lose — at least temporarily — their insulating or semi-permeable properties; hence a certain loss of material results, as indicated by the loss of pigment; at the same time the muscle cells undergo intense stimulation, and a well-marked toxic or generally disorganizing action sets in. These last two effects appear to be direct consequences of the change in permeability; this is indicated by the close parallelism which

exists between the degree of the permeability-increasing action of salt solutions and both their stimulating and toxic action. Certain substances decrease or prevent the permeability-increasing action of the pure salt solution; among those are not only various neutral salts — *e. g.*, those of calcium and magnesium — but also, as I have recently found, a large number of lipoid-solvent anæsthetics; at the same time they decrease or prevent the immediate stimulating action of the solution and lessen its toxic action. This parallelism indicates that the semi-permeable surface films or plasma membranes of the cells form the *locus* both of the stimulating and of the toxic action of the salt solution, and that the above substances exert their anti-stimulating and antitoxic action by altering the plasma membranes in such a way as to decrease or prevent the permeability-increasing action on which both effects depend.

Direct observation thus indicates that modification of surface structures forms the basis of certain characteristic physiological effects produced by neutral salts and anæsthetics in these organisms. The pure salt solution causes visible changes in the physical state of the colloidal filaments and membranes at the cell surfaces. These effects, in so far as they are irreversible, are toxic. Thus it is immediately apparent why pure sodium chloride solution arrests ciliary movement; the colloids of the tissue are so altered as to *interrupt the continuity* of the contractile filaments; the latter then necessarily cease movement and undergo breakdown. In such a case the effect is essentially irreversible. Presumably similar changes take place in the plasma membranes of the cells; obviously any such interruption in the continuity of the membranes would necessarily involve marked increase of permeability. The case of the cell, however, differs from that of colloidal filaments like cilia in that there always remains the possibility that the continuity of the surface film may be restored by deposition of materials from the protoplasm. Possibly this is why the destructive action of the pure salt solution on the cells (*e. g.*, muscle cells) of this organism is more gradual than on the cilia, so that some recovery is possible after relatively prolonged exposure. It seems clear that the preservation of the continuity and semi-permeability of the surface films is essential to the continuance of normal vital processes; hence the presence of substances that favor this condition protects the cell against injury by toxic substances or other destructive agencies.

II. EXPERIMENTAL.

In the experiments described in the present and preceding papers I have tested the action of a variety of anæsthetics in modifying the above-described stimulating, permeability-increasing, and toxic action of the pure isotonic NaCl solution. The substances used include the following: a series of alcohols, methyl, ethyl, n-propyl, iso-propyl, n-butyl, n-amyl, n-capryl; chloretone; a series of esters, — ethyl acetate, propionate, butyrate, valerianate, nitrate; methyl, ethyl, and phenyl urethanes; various normal and substituted hydrocarbons, — carbon tetrachloride, chloroform, nitromethane, acetonitrile, benzol, toluol, xylol, phenanthrene, naphthalene; and a number of miscellaneous compounds, — ethyl ether, chloral hydrate, chloralose, paraldehyde, phenyl urea, acetanilide, phenacetin, methacetin. The action of certain inorganic salts (as of magnesium and calcium) and of isotonic sugar solution falls partly into the anæsthetic category, but will not be considered in the present paper.

In these experiments the solution, 0.55 m. NaCl, containing the anæsthetic in known concentration, is added suddenly to a mass of larvæ which have been collected in a watch glass or in the interior of a small flask. The immediate effects of the solution on muscular and ciliary movement and the degree of revival in sea water after known periods of exposure, can be readily determined and compared with the corresponding effects of the control solution (pure 0.55 m. NaCl).

In general it has been found that all anæsthetics — provided their solubility in salt solution is sufficient — show the following effects in common. In concentration above certain maxima they cause rapid cytolysis, and reinforce the disintegrative action of the pure salt solution. In weaker solutions, within a certain range of concentrations characteristic for each anæsthetic, they exhibit a definite anti-stimulating and anti-cytolytic action. The degree of this action varies with different anæsthetics, but is constant and characteristic for any particular compound. Usually the muscular contraction on the first contact with the anæsthetic-containing solution is much less intense and prolonged than in the pure salt solution, and in many cases it is completely or almost completely prevented. The immediate loss of pigment is also usually decreased, and in many cases en-

tirely prevented; the degree of this effect shows a close parallelism with that of the decrease of stimulation. Ciliary movement is almost always decidedly prolonged — in favorable cases for several hours. If after a certain stay in the solution the larvæ are returned to sea water, they are found to recover muscular contractility more rapidly and completely than after a similar exposure to pure 0.55 m. NaCl. The general disintegration produced by the salt is also lessened or retarded in the presence of the anæsthetic, and the larvæ remain alive longer after return to sea water. The anæsthetic thus shows a definite protective or antitoxic action similar to that of calcium chloride and other salts; this effect is highly characteristic and has been shown without exception by every substance so far tried which exhibits definite anæsthetic action in sea water.

The concentrations most favorable for the prevention of the stimulating and cytolytic effects of the salt solution correspond closely with those which produce typical anæsthesia in normal larvæ in sea water. Hypo-anæsthetic concentrations always fail to prevent, though they may decrease, the immediate stimulation and loss of pigment; but in most cases, unless too low, they exert a distinct protective or antitoxic action, — *i. e.*, they retard the disintegrative or cytolytic action of the salt solution, and on return to sea water from such solutions recovery is found to be more complete than after similar exposure to the pure salt solution. Another remarkable and characteristic effect of anæsthetics in hypo-anæsthetic concentrations is to *prolong* the period during which muscular contractions continue in the salt solution; in pure 0.55 m. NaCl all contractions typically cease within four minutes or less, and the larvæ remain quite motionless during their further stay in the solution; but in the presence of the anæsthetic slight contractions often continue steadily — in many cases for half an hour or more. This effect recalls the stimulating or sensitizing action so characteristic of anæsthetics in weak solution; but it seems rather to be the expression of a certain protective or antitoxic action which enables the tissue partly to preserve its normal properties even in the otherwise strongly toxic pure salt solution. The presence of traces of calcium chloride has a similar effect.

ACTION OF SPECIAL ANÆSTHETICS.

Alcohols. — All of the above-named alcohols in favorable concentrations decrease or prevent the initial stimulating and permeability-increasing action of isotonic sodium chloride solution, protect the cilia against immediate breakdown, thus prolonging their activity, and retard the general toxic action. The optimum concentrations for the immediate anti-stimulating and anti-cytolytic actions are in general the same; as already said, they correspond closely with the concentrations which produce typical anæsthesia in sea water. These concentrations show the usual progressive decrease with increase in the molecular weight of the alcohol. In physiologically equivalent solutions the different alcohols modify the action of the salt solution in essentially the same manner. If the proportion of alcohol is favorable the larvæ show no immediate loss of pigment and little or no stimulation; either no evident muscular contraction occurs on the first contact with the solution — the larvæ merely ceasing movement and remaining relaxed and motionless — or the resulting contraction is slight or moderate and followed by immediate relaxation and complete cessation of movement. In such solutions the cilia typically continue their movement. In concentrations of alcohol somewhat higher than optimum the cytolytic action of the solution is eventually accelerated, though there may be typical protective action at first. In still stronger solutions the cilia cease at once, and cytolysis is rapid from the first. There are certain specific differences of toxicity between the different alcohols; propyl and butyl alcohols have shown the most favorable, *i. e.* most completely reversible, protective action, while capryl alcohol always shows a marked specific toxicity, even in concentrations that at first appear highly favorable. Chloretone, which may be classed with the alcohols, is remarkably rapid and complete in both its anti-stimulating and anti-cytolytic action, as already described.⁷

The following is a summary of the observations made with alcohols. The control solution with which comparison is made is always the pure 0.55 m. NaCl.

⁷ This Journal, 1912, xxix, p. 387.

1. **Methyl alcohol.** — A solution of 10 vol. per cent in 0.55 m. NaCl causes a well-marked immediate contraction (though less than the control) followed by instant relaxation; no further muscular contractions are seen; the cilia remain active for half an hour or more. A little pigment is liberated at first, but much less than in the control.

Effect of return to sea water. — After forty-five minutes in the solution the larvæ recover muscular contractions much more completely than in the control; the cilia also revive in part; after three hours' exposure recovery is also greater than in the control, but the difference is less; after six hours there is no recovery either in the solution or in the control.

In 5 vol. per cent solution strong stimulation and well-marked exit of pigment occur essentially as in the control. The cilia are protected only slightly, and most cease movement within five minutes. Slow muscular contractions continue for an hour or more in this solution.

Effect of return to sea water. — The protection is at first less marked than in the 10 per cent solution, but lasts longer. After intervals of three and six hours active contractions return in sea water; even after twenty-two hours in the solution many larvæ recover slightly.

Anæsthetic concentration in sea water. — This is *ca.* 10 vol. per cent. There is no muscular movement in this solution and recovery is well marked after eighteen hours. In 5 vol. per cent solution contractions continue actively.

2. **Ethyl alcohol.** — *a.* 10 vol. per cent. There is a slight muscular contraction at first and no immediate loss of pigment. The cilia cease at once. After two and one-half hours cytolysis is well marked.

Return to sea water. — In larvæ transferred after twenty-three minutes and forty-five minutes the cilia revive and well-marked contractions return; after two and one-half hours there is no revival.

b. 5 vol. per cent. There is moderate contraction at first with instant relaxation. No loss of pigment is evident. Cilia continue for some time. The muscular anæsthesia is not quite complete; occasional slight contractions persist for some time.

Return to sea water. — Larvæ returned after forty-five minutes recover almost completely; some show heliotropic swimming, though the direction of the response is reversed (negative). After two and one-quarter and five hours active contractions return, much greater than the control. A fair proportion recover contractility after twenty-one and one-half hours in the solution.

Anæsthesia in sea water. — In sea water with 6 vol. per cent alcohol

muscular anæsthesia is practically complete. The effect is perfectly reversible. In 5 vol. per cent. slight contractions continue; 10 vol. per cent is rapidly toxic.

3. *n*-propyl alcohol. — Solutions in 0.55 m. NaCl of the following concentrations were used: 5, 4, 2.5, 2, 1.25, 1, 0.63, and 0.5 vol. per cent. In general the effects were similar to those seen with ethyl alcohol, but somewhat more favorable. In the stronger solutions down to 2 vol. per cent (inclusive) there is almost no immediate stimulation or loss of pigment. In 1.25 and 1 vol. per cent stimulation is stronger and there is slight immediate loss of pigment (< control); in 0.63 and 0.5 vol. per cent stimulation and exit of pigment are well marked. The protective action on cilia is decided down to 2 vol. per cent (in 2.5 vol. per cent solution some ciliary movements remained after seventeen hours); below this it is less distinct or absent. In 1.25 per cent and weaker solutions muscular anæsthesia is incomplete and contractions continue for some time — in some cases for more than an hour.

Return to sea water. — In the 5 vol. per cent solution the larvæ, though protected at first, die within an hour; in the 4 per cent solution cytolytic changes appear within one and a half hours. Revival after one hour in this solution is greater than in the control. Larvæ from the 2.5 per cent solution show a remarkably prompt and perfect recovery after an exposure of forty minutes, and many recover helio-tropism (mostly negative); the same was observed with the 2 per cent solution after one hour's exposure, and with the 1.25 per cent after thirty minutes. In the weaker solutions protection is at first less complete — *i. e.*, the initial injury is greater — though it remains evident after longer periods of exposure. After seventeen hours' exposure larvæ from 2.5, 1.25, and 0.63 vol. per cent showed good revival of contractions; the same was true of 1 and 0.5 per cent solutions after twenty-two hours' exposure.

Anæsthetic action in sea water. — The minimum concentration is between 2 and 3 vol. per cent; arrest of contractions is not quite complete in 2 vol. per cent solution.

4. Isopropyl alcohol. — This was used in the concentrations 4, 3, 2, and 1 vol. per cent, and showed essentially the same action as the normal alcohol, although in equivalent concentrations both its anæsthetic and toxic actions appeared somewhat weaker. In 4 and 3 vol. per cent solutions immediate stimulation is slight and there is no loss of pigment; in 2 vol. per cent stimulation and loss of pigment are well marked, though less than in the control; in 1 vol. per cent there is little difference from the control. Protective action both on muscle and cilia is

well marked, — best in 3 vol. per cent and relatively slight in 1 vol. per cent.

5. *n*-butyl alcohol. — The following solutions were used: 4, 2.5, 2, 1.25, 1, 0.62, and 0.31 vol. per cent. The same effects were observed as with propyl alcohol, but the physiologically equivalent concentrations were in all cases lower: 4 vol. per cent causes rapid cytolysis and breakdown of cilia; 2.5 vol. per cent causes rapid cytolysis within fifteen minutes, but prevents the immediate stimulation and loss of pigment; 2 vol. per cent prevents stimulation and loss of pigment and preserves ciliary movement for an hour or more, but is fatal within two hours. In 1.25 and 1 vol. per cent solutions the protective and anti-stimulating actions are best marked; there is little or no immediate stimulation and no exit of pigment, and ciliary movement lasts for an hour or more; 0.62 vol. per cent acts similarly; a few cilia remain active after eighteen hours in this solution, but muscular anæsthesia is not quite complete; in 0.31 vol. per cent stimulation and pigment exit are well marked, though less than the control; the cilia cease in a few minutes, while muscular contractions continue.

Return to sea water. — Larvæ returned to sea water after fifty-five minutes in 2.5 vol. per cent alcohol showed no revival. After a similar stay in 1.25, 1, and 0.62 vol. per cent recovery of contractions was prompt and complete, and many larvæ showed a return of the normal positive phototaxis (especially with 1.25 and 1 vol. per cent); after 0.31 vol. per cent recovery of contractions was slower and less complete and the cilia failed to revive. Muscular contractions reappeared after eighteen hours in the 1 and 0.62 and 0.31 per cent solutions (best with 0.62 per cent), but not in 1.5 per cent (no revival in control).

Anæsthetic concentration in sea water. — The minimum is *ca.* 0.8 vol. per cent, which causes complete muscular anæsthesia without arresting the cilia.

6. *n*-amyl alcohol. — The following solutions were used: 1, 0.5, 0.4, 0.25, 0.2, 0.13, and 0.1 vol. per cent. In 1 vol. per cent solution cytolysis is rapid. In 0.5, 0.4, 0.25, and 0.2 vol. per cent the initial stimulation is slight and there is no loss of pigment; in 0.13 per cent the initial stimulation and pigment exit are well marked, though less than in the control; 0.1 vol. per cent shows little difference from the control. In 0.5, 0.4, 0.25, and 0.2 vol. per cent breakdown of cilia is prevented and ciliary movement is prolonged for about an hour; in 0.13 and 0.1 per cent the cilia cease almost as soon as in the control. Muscular anæsthesia is incomplete below 0.25 vol. per cent, and slight contrac-

tions continue in these solutions (0.2, 0.13, 0.1 per cent) for some time.

Return to sea water. — One vol. per cent is quickly destructive. In the case of the other solutions larvæ returned within one hour to sea water recovered much more completely than the control, but phototaxis was not renewed; 0.1 vol. per cent is the least favorable. With prolonged exposure (eighteen hours or more) larvæ from 0.5 vol. per cent showed no revival, those from 0.4 vol. per cent revived only slightly (again the stronger solutions, though showing better protection at first, are less favorable than the weaker when the exposure is prolonged); larvæ from 0.25, 0.2, and 0.13 vol. per cent showed well-marked renewal of contractions, and from 0.1 per cent slight renewal. There was no recovery in the control.

Anæsthetic concentration in sea water. — The minimum for complete muscular anæsthesia is *ca.* 0.25 vol. per cent.

7. *n*-capryl alcohol. — Capryl alcohol ($C_8H_{17}OH$) is only slightly soluble in water (1 part in *ca.* 2000). The solutions used (in 0.55 m. NaCl) were: saturated (*ca.* 0.05 per cent), three-fourths saturated, half saturated, and three-eighths, one-fourth, and one-eighth saturated. In the saturated solution the immediate contraction and loss of pigment are completely prevented, but within fifteen minutes cytolysis sets in; in half and three-fourths saturated there is also little or no immediate stimulation and no exit of pigment, and the cilia continue their activity (in some larvæ for almost three hours); in the three-eighths saturated the initial contraction is well marked, there is slight loss of pigment, and the cilia cease soon; in the one-fourth and one-eighth saturated solutions the initial contraction and pigment exit are pronounced, and protection to cilia is slight or absent; in these two solutions muscular contractions continue for some time.

Return to sea water. — Protective or antitoxic action is well marked at first in all solutions except the saturated. After three hours in the half, three-eighths, and one-fourth saturated solutions the revival of contractions in sea water was decidedly greater than in the control; one-eighth saturated showed relatively little action. After six hours' exposure there was slight revival in larvæ from the half and three-eighths saturated solutions, but not from the others. There was no revival after twenty-two hours. The strong specific toxicity of this alcohol asserts itself with the longer exposures, although typical antitoxic effects are plainly evident at first.

Anæsthetic concentration in sea water. — The optimum anæsthetic concentration is *ca.* one-third saturated; half-saturated solutions are

fatal within an hour, while with one-fourth saturation muscular anæsthesia is not quite complete. This alcohol has a high specific toxicity; after eighteen hours all of the larvæ in the one-third saturated and most in the one-fourth saturated were found dead.

Fatty acid esters. — To save space a less detailed account of the observations with these compounds will be given. Their general action is similar to that of the alcohols, but the toxicity is greater (due probably to hydrolysis) and the protective action less. The four ethyl esters, acetate, propionate, butyrate, and valerianate, were used. As with the alcohols, the favorable anæsthetic and protective concentrations decrease progressively with increase in the molecular weight. In one respect these esters — especially the higher members — differ from most of the anæsthetics investigated in these experiments in showing a well-marked specific or selective action on the pigment-containing cells of this organism. Solutions of ethyl butyrate and valerianate in sea water, in favorable anæsthetic concentrations, extract considerable pigment from the larvæ; ethyl propionate shows this action to a less degree, while the acetate anæsthetizes without loss of pigment. In correspondence with this peculiarity the two higher esters do not prevent the immediate loss of pigment in 0.55 m. NaCl, although they inhibit stimulation and retard toxic action in the same manner as the others.

Action of esters in 0.55 m. NaCl. — 2.5 vol. per cent solutions of ethyl acetate are rapidly fatal. In 2, 1.5, and 1 vol. per cent solutions there is only slight immediate stimulation and little or no loss of pigment, and ciliary movement lasts decidedly longer than in the pure salt solution. In 0.5 and 0.25 vol. per cent solutions there are strong contraction, marked loss of pigment, and immediate cessation of ciliary movement; slow muscular contractions continue in these solutions for some time.

Transfer to sea water. — Well-marked protective action is shown by all of the above solutions from 2 vol. per cent down; but in the stronger solutions, 2 and 1.5 vol. per cent, it lasts for only a short time; after a few hours in these solutions there was no recovery. After four hours in the 1, 0.5, and 0.25 per cent solutions revival of contractions was much more complete than in the control (0.5 vol. per cent proved the most favorable).

Anæsthetic concentration in sea water. — Solutions of 1 to 1.5 vol. per cent produce complete typical muscular anæsthesia in sea water.

Similar effects are seen with the other three esters, the physiologically equivalent concentrations decreasing with increase in the molecular weight. The anæsthetic concentrations in sea water are approximately as follows: propionate, 0.4 to 0.5 vol. per cent; butyrate, 0.13 to 0.25 vol. per cent; valerianate, 0.13 to 0.08 vol. per cent. Solutions of these concentrations in 0.55 m. NaCl show typical anti-stimulating and anti-cytolytic action, and prevent the immediate breakdown of cilia; stronger solutions cause cytolysis, while in weaker solutions stimulation and ciliary breakdown occur essentially as in the pure solution, and muscular contractions are prolonged. Butyrate and valerianate are relatively inefficient in preventing the immediate exit of pigment, though showing typical anti-stimulating and antitoxic action.

Other esters examined include ethyl nitrate and the urethanes (methyl, ethyl, and phenyl). The following is a brief account of the observations with these compounds.

Ethyl nitrate. — Solutions (in 0.55 m. NaCl) of the concentrations 0.75, 0.5, 0.35, and 0.25 vol. per cent were used. This ester shows strongly marked anti-stimulating and anti-cytolytic action. In all of the above concentrations the immediate stimulation and loss of pigment are slight or absent, and ciliary movement continues; in 0.75 vol. per cent a gradual cytolysis begins within a few minutes, and the larvæ show little or no recovery in sea water after an hour's exposure; in the 0.5 per cent solution the anæsthetic and protective actions are typical and lasting; in 0.35 and 0.25 per cent muscular anæsthesia is only partial, and slow contractions continue for some time.

Return to sea water. — After half an hour in the above solutions all of the larvæ recovered much more completely than in the control, and a certain proportion of those from 0.5 vol. per cent showed negative phototaxis; of the larvæ transferred to sea water after five hours in the last three solutions, all showed active contractions next day, while all from the control and from 0.5 vol. per cent were dead.

Anæsthetic concentration in sea water. — One vol. per cent causes rapid cytolysis; 0.35 and 0.5 vol. per cent produce typical reversible anæsthesia; in 0.25 vol. per cent muscular anæsthesia is incomplete, and slight contractions continue.

Ethyl urethane. — In my former paper experiments with this ester were described, but the strongest solution used, 0.6 per cent, was insufficient to prevent stimulation and loss of pigment in 0.55 m. NaCl. In higher

concentrations urethane shows this action typically. In 4 per cent and 2 per cent solutions immediate loss of pigment is prevented, stimulation is greatly diminished and the cilia continue activity — in the 2 per cent solution for an hour or more. In 1 per cent urethane there are considerable immediate stimulation and loss of pigment, — though both are less than in pure 0.55 m. NaCl — and the cilia cease soon.

Return to sea water. — With all of the above solutions recovery is at first (within half an hour) much greater than in the control. The 4 per cent solution is gradually destructive, and after four hours no revival was seen. With the 2 per cent solution recovery after five hours was decidedly greater than in the control. Weaker solutions also show well-marked protective action, as described in my former paper.

Anæsthetic concentration in sea water. — One per cent urethane produces only partial anæsthesia. In the 2 per cent solution anæsthesia is almost, and in 3 per cent quite, complete. Four per cent is toxic; the larvæ fail to revive after four hours' exposure, and later undergo disintegration.

Methyl urethane. — Comparatively few observations were made with this compound. Its action is weaker than that of ethyl urethane. In 5 vol. per cent solutions in 0.55 m. NaCl stimulation and loss of pigment are well marked, but the cilia are protected and remain active for about an hour. The 2 per cent solution shows an immediate action like that of pure 0.55 m. NaCl.

Return to sea water. — Both of the above solutions show well-marked antitoxic action, especially at first. The 5 per cent solution is fatal within four hours.

Anæsthetic concentration in sea water. — Two per cent solutions show no permanent anæsthetic action; and phototactic swarming, partly negative, is renewed in a few minutes; 5 per cent solutions anæsthetize the musculature, but the revival after four hours' exposure is imperfect.

Phenyl urethane. — Saturated solutions of this compound in 0.55 m. NaCl exhibit typical anti-stimulating and anti-cytolytic effects. There is almost no loss of pigment, and only slight immediate contraction, followed by instant relaxation; the cilia remain active. This solution, however, is somewhat too strong, and cytolysis sets in within two hours.

Return to sea water. — Recovery after thirty minutes' exposure is prompt and complete (much better than in the control). After cytoly-

sis has begun in the solution the larvæ fail to revive in sea water. Observations with weaker solutions were not made.

Anæsthetic concentration in sea water. — Muscular anæsthesia is typical and complete in saturated solutions, but incomplete in half-saturated. The optimum is evidently intermediate; in saturated solutions the larvæ die within a few hours; in half-saturated they continue to live, but show slight contractions.

Hydrocarbons. — Experiments with chloroform, benzol, xylol, and toluol were briefly described in my former paper. The three cyclic hydrocarbons differ in their solubility in water, benzol being the most and xylol the least soluble; the physiological effects of their saturated solutions vary correspondingly; benzol produces complete reversible muscular anæsthesia in half-saturated solutions in sea water, and cytolysis in saturated; for toluol half-saturation is insufficient for anæsthesia, which, however, is typical in saturated; xylol in saturated solution produces only partial anæsthesia. In 0.55 m. NaCl all three fail to prevent immediate stimulation and loss of pigment, although they show well-marked antitoxic effects in concentrations corresponding to anæsthetic or slightly hypo-anæsthetic solutions in sea-water. Chloroform shows in NaCl solutions typical anti-stimulating and anti-cytolytic action in concentrations corresponding closely to those causing typical anæsthesia in sea water.

Observations were made last summer with three other substituted methanes, nitromethane, acetonitrile, and carbon tetrachloride.

Nitromethane (CH_3NO_2). — This compound shows highly characteristic action. In 5 vol. per cent solutions in 0.55 m. NaCl the larvæ contract strongly at first but without immediate loss of pigment; the cilia ceasesoon, and cytolytic action is evident within fifteen minutes. In 2.5 vol. per cent solution the initial contraction is moderate and followed by rapid relaxation and cessation of movement; there is no trace of pigment exit, and the cilia continue actively (in one experiment for some hours). In 1.5 vol. per cent solution there are prolonged contraction and well-marked loss of pigment, the cilia cease soon, and muscular contractions continue for a half-hour or more.

Return to sea water. — This is ineffective with the 5 vol. per cent solution after fifteen minutes; but if returned to sea water within five minutes the larvæ recover much more rapidly and completely than the control. Larvæ brought from 2.5 vol. per cent to sea water

within half an hour revive completely, and many show a return of phototaxis (both positive and negative); after three or more hours revival is less complete. In 1.5 per cent solutions the musculature and general organization are well protected for some hours, but not the cilia. The anæsthesia produced by nitromethane is very persistent, and revival in sea water is correspondingly gradual, but unusually complete if the exposure is not too prolonged.

Anæsthetic concentration in sea water. — Five vol. per cent solutions in sea water cause rapid cytolysis. In 2.5 vol. per cent anæsthesia is complete and normal.

Acetonitrile (methyl cyanide: CH_3CN). — This compound resembles nitromethane in its action, although the effective concentrations are higher. In 10 vol. per cent solutions cytolysis is rapid. In 5 vol. per cent solutions the immediate stimulation is slight, there is no loss of pigment, and the cilia remain active (for half an hour or more).

Return to sea water. — Recovery after half an hour in the 5 vol. per cent solution is much prompter and more complete than in the control, and many larvæ regain their phototaxis. After *ca.* three hours active contractions are renewed (much more active than in the control); after seventeen hours in the 5 per cent solution no revival was seen.

Anæsthetic concentration in sea water. — Anæsthesia is typical and complete in *ca.* 5 vol. per cent solutions, 10 vol. per cent solutions are rapidly destructive, and in 2.5 vol. per cent solution muscular anæsthesia is incomplete.

Carbon tetrachloride. — In its action this compound closely resembles benzol. In saturated solution in 0.55 m. NaCl it is quickly destructive. In one-half, one-third, and one-fourth saturated solutions immediate stimulation and loss of pigment are pronounced, as in the control; in the half-saturated solution ciliary movement is somewhat prolonged.

Return to sea water. — Well-marked protective action was observed in the one-third and one-fourth saturated solutions after seventeen hours' exposure; the half-saturated solution was fatal with this length of exposure, though it showed distinct protective action at first.

Anæsthetic concentration in sea water. — The saturated solution is quickly toxic. The half-saturated produces typical muscular anæsthesia, but causes some loss of pigment. In the one-third saturated solution anæsthesia is incomplete.

Phenanthrene and naphthalene. — These compounds are too insoluble to produce any appreciable effects in sodium chloride solution. The saturated solutions in sea water show little or no anæsthetic action;

the only evident effect is a disturbance of the heliotropic swarming which disappears in naphthalene-saturated sea water within a minute; later, however, a slight negative phototaxis shows itself. In sea water saturated with phenanthrene the normal heliotropic swarming continues almost unaffected at first, but later the sense of the response is largely reversed; within one hour about half of the larvæ show a negative phototaxis; the rest remain positive.

Chloral hydrate. — Observations with this compound were described in my former paper. Its action is apparently too gradual to prevent the immediate stimulation and loss of pigment, though in favorable concentrations it retards the disintegration caused by pure NaCl solutions and shows well-marked antitoxic action. In sea water muscular anæsthesia comes on gradually, but completely in solutions of 0.12 to 0.2 per cent; in 0.08 per cent there is partial inhibition of muscular movements, incomplete after eighteen hours. Concentrations of 0.5 per cent and upward are toxic.

Chloralose. — This compound is too insoluble to show well-marked action. In saturated solutions in 0.55 m. NaCl the initial stimulation and loss of pigment are similar to the control, and the cilia cease movement at once; muscular contractions continue for some time in this solution (an effect like that of other hypo-anæsthetic solutions), and there is a distinct general protective action.

Return to sea water. — Transfer to sea water after an hour in the above solution is followed by decidedly more complete revival than in the control; after twenty hours' exposure there was some revival in sea water, and the general disintegration was less than in the control.

Anæsthetic action in sea water. — In chloralose-saturated sea water the larvæ show normal heliotropic swarming at first; but a well-marked anæsthetic action asserts itself in time; in an hour the muscular contractions have almost ceased. Return to sea water after six hours is followed by complete revival.

Paraldehyde. — In 3 vol. per cent and 4 vol. per cent solutions in 0.55 m. NaCl the immediate stimulation and loss of pigment are prevented or greatly decreased, and ciliary breakdown is checked. Prolonged action of these solutions is injurious. The protective action is well marked at first (up to ten or fifteen minutes), but the larvæ die within an hour. No observations were made with weaker solutions. Anæsthesia in sea water is complete in 2.5 vol. per cent solutions; 5 vol. per cent is too concentrated and causes rapid cytolysis.

Acetanilide. — In saturated solutions of acetanilide in 0.55 m. NaCl there is only slight stimulation with almost no immediate loss of pig-

ment, and ciliary movement is prolonged. Within an hour cytolytic action sets in. Protection is well marked at first; larvæ transferred to sea water after ten minutes revive promptly and completely, many renewing their heliotropic swarming; after half an hour's exposure contractions also revive much more completely than in the control; but an hour's exposure is fatal. The saturated solution in sea water produces typical complete muscular anæsthesia, with continuance of ciliary movement. This concentration is higher than the optimum and causes injury or death with prolonged exposure.

Phenyl urea. — Saturated, three-fourths saturated, and half-saturated solutions of this compound in 0.55 m. NaCl fail to prevent the immediate stimulation and loss of pigment; in the first two concentrations ciliary movement is prolonged; in the half-saturated solution the cilia cease soon, and muscular contractions continue for some time (the usual effect of hypo-anæsthetic solutions).

Return to sea water. — Transfer to sea water after an hour in the above solutions is followed by more complete revival than in the control (best with the half-saturated solution). After eighteen hours' exposure no revival was seen.

Anæsthetic concentration in sea water. — The saturated solution causes contraction and loss of pigment followed by complete muscular anæsthesia. This solution is fatal within a few hours. In half-saturated solutions anæsthesia is incomplete.

Phenacetin and methacetin. — These compounds are too insoluble to influence the action of 0.55 m. NaCl. The saturated solutions in sea water have no apparent anæsthetic action; the larvæ show the usual strong positive phototaxis in such solutions; the only effect noted was an increased vigor of ciliary movement in the phenacetin solution.

III. GENERAL DISCUSSION.

On reviewing the above observations certain fundamental uniformities in the action of the different anæsthetics are seen. In the first place it appears that those strengths of solution which in sea water produce typical muscular anæsthesia are the most effective in preventing both the immediate strong muscular contraction in the sodium chloride solution and the immediate exit of pigment from the body cells. This parallelism is highly characteristic, and affords strong indication that the primary basis of both stimulating and pigment-

liberating actions is the same, namely, a rapid and well-marked increase in the permeability of the limiting membranes of the cells. In all those cases where the anæsthetic plainly increases the resistance of the pigment-containing cells to the permeability-increasing action of the salt solution it is found that the muscle cells also become resistant or irresponsive to its stimulating action. In the converse group of cases (*e. g.*, chloral hydrate, chloralose, phenyl urea, hydrocarbons) where the anæsthetic fails to prevent the immediate strong stimulation on contact with the solution — apparently because of a too gradual action — it also fails to prevent the exit of pigment. With most anæsthetics that produce prompt and typical anæsthesia both effects undergo a simultaneous and closely parallel decrease or prevention in solutions of appropriate concentration. Secondly, the general cytolytic or toxic action of the salt solution is also in all cases decreased or retarded in the presence of the anæsthetic, so that the larvæ on return to sea water recover their normal movements and behavior more completely than after a similar exposure to the pure solution. Substances of this class, in anæsthetic or moderately hypo-anæsthetic concentrations, thus exert a definite protective or antitoxic action; this is best marked in those cases where the initial stimulation and loss of pigment are entirely prevented, but it is shown also, though in general to a less degree, even where no such immediate antagonistic action is apparent.

The evidence at present available indicates that the anæsthetic produces these various effects by imparting to the plasma membranes or other surface structures (as cilia) an increased resistance to the alterative or disintegrative action of the pure salt solution. This it does presumably by altering the physical state of the lipid components of these structures; the permeability-increasing and other associated effects ordinarily caused by the salt solution — including stimulation, and toxic action — are thus checked or prevented, for a time at least. It might be urged that the above observations contain no direct proof that changes in the permeability of the pigment-cell membranes form any index of similar changes in the membranes of the irritable elements. This may be admitted, although the conditions controlling the permeability of cells have been shown to be so widely uniform that any objection thus based seems rather formal than real. There is, however, independent evidence that the anæsthetic acts alike on the

pigment cells and on the contractile elements in the fact that it protects both simultaneously against the toxic or destructive action which the pure salt solution exerts on both. The initial action of the pure salt solution on pigment cells and on muscle cells thus appears to be essentially the same; and since clear evidence exists in the case of the pigment cells that this action consists in a marked and rapid increase of surface permeability, it is to be inferred that in the muscle cells also a similar change takes place. If the anæsthetic demonstrably prevents this rapid increase of permeability in the one case, it may be assumed to do so in the other. We infer, therefore, that it prevents stimulation and toxic action by preventing the rapid increase of permeability essential to both processes.

Increase of permeability in the pigment cells, as shown by exit of pigment, appears in the great majority of instances to be a reliable index of the occurrence of similar changes in the irritable elements. There is, however, even at this early stage, considerable differentiation among the cells of *Arenicola* larvæ, and the above rule appears to be not invariable; certain anæsthetics, *e. g.*, ethyl butyrate, ethyl valerianate, and chloroform in higher concentrations, extract considerable quantities of pigment in sea water, although causing anæsthesia without marked preliminary stimulation. A certain selective affinity of different cells for different anæsthetics seems thus already to exist in these organisms. The existence of such cases does not alter the general rule; the above substances, though attacking the pigment cells, show typical anti-cytolytic or protective — as well as anæsthetic — action on the muscle cells themselves. In the great majority of instances the degree of immediate exit of pigment in sodium chloride solutions containing anæsthetics — or antitoxic salts like calcium or magnesium chloride — exhibits a close parallelism with the intensity of the stimulating action on the musculature.

These observations have an obvious bearing on the general problem of the nature of the critical or primary change in the stimulation of irritable tissues in general. That a change of electrical polarization at the semi-permeable membranes of the irritable elements is the essential change in electrical stimulation has, since Nernst's study of this problem, been very generally recognized by physiologists. It seems improbable that a polarization change at the limiting membranes can be an exclusive peculiarity of the electrical form of stimulation;

other forms of stimulation ought theoretically to be associated with a similar effect, since all stimuli elicit the same essential response in an irritable tissue. If this inference is correct, there should be a physiologically produced change in the polarization of the limiting membranes during normal stimulation; and the electrical variation of stimulation is evidence that this is in fact the case. There is already wide agreement among general physiologists that the state of excitation is associated with a variation of electrical polarization at the limiting membranes. But apparently little agreement exists as yet with regard to the nature of the process that immediately conditions this change of polarization. The latter might conceivably be a chemical effect, due *e. g.* to an increased rate of oxidations, altering the ionic content of the protoplasm adjoining the membrane independently of alterations in the membrane itself; or it might be the expression of purely physical changes in the membrane, *e. g.*, changes of permeability, affecting directly its influence on ionic diffusion and so its polarization; or possibly both kinds of factors might be concerned. Any experimental evidence that the properties of the membrane are in fact altered during stimulation has thus an important bearing on the general problem of stimulation.

The above observations show that correlated with the loss of irritability is a definite alteration in the character of the limiting membranes. These become more resistant to changes in their permeability as the irritable tissue becomes more resistant to stimulation. This fact supports the view that in stimulation an increase in the permeability of the membranes is essential. Apparently normal irritability is associated with a certain state of the plasma membrane, in which the latter undergoes increase of permeability under certain conditions — especially changes in its electrical polarization — with a certain characteristic degree of readiness. During anæsthesia this readiness is decreased. The inexcitable condition produced by anæsthetics thus corresponds to one in which permeability is altered with difficulty.

Other facts and considerations, in part already well known, support this view. The most constant features of the stimulation process seem to be (1) the existence of a negative electrical variation, and (2) the existence of an inexcitable or so-called refractory period during a certain time after the beginning of the response. The time relations

of these two apparently quite different effects appear to correspond closely.⁸ The primary change in stimulation must therefore be of such a kind as to account for both. The only hypothesis — so far as I know — which gives a theoretically satisfactory explanation of this conjunction is that which refers *both* changes to a temporary and well-marked increase in the general permeability of the limiting membranes of the irritable elements. Such an increase would deprive the membranes of their capability of becoming the seat of an electrical polarization; it would therefore account for the observed fall in the potential difference between the interior and exterior of the irritable elements during stimulation, as well as for the inability of the tissue to respond to electrical excitation at this time, since this response depends (as Nernst and his successors have shown) on the production of polarization effects at the semi-permeable membranes, and during stimulation the necessary semi-permeability is lost. In addition to these theoretical grounds for regarding an increase of permeability as a necessary and primary condition of stimulation, there are various direct observations which point in the same direction. I have several times reviewed these observations and need refer to them here only briefly. Perhaps the most unequivocal evidence of this kind is the fact that in motile plant cells (of *Mimosa*, *Dionæa*, etc.) definite osmotic effects accompany stimulation, and indeed form the essential condition of the response. The turgor of these cells is temporarily lost; and since turgor depends on the semi-permeability of the plasma membranes, loss of turgor is a direct indication of loss of semi-permeability. No satisfactory substitute for this simple and adequate explanation has, to my knowledge, as yet been proposed. It is unlikely that the stimulation process differs in its essential nature in the two groups of organisms; and the above-described experiments with *Arenicola* larvæ indicate clearly that in the irritable tissues of animals the limiting membranes also undergo temporary increase in permeability during stimulation. There is also strong evidence that narcotic action in plants is dependent on a change in the character of the membranes identical with that just described. Anæsthetics prevent the characteristic response of *Mimosa* and plants with similar mechanisms; *i. e.*, the ether-impregnated plasma membranes no longer undergo rapid increase of permeability

⁸ Cf. TAIT: Quarterly journal of experimental physiology, 1910, iii, p. 221.

on stimulation, but retain their semi-permeability, so that turgor remains unaltered.⁹

The above-described protective or antitoxic action of anæsthetics resembles in all essential respects that of salts like calcium or magnesium chloride, and both seem primarily due to the influence of the antitoxic agent on the plasma membranes or other surface structures concerned (cilia). The suggestion that in antagonistic salt-actions the antitoxic salt acts by modifying the permeability of the membranes to the toxic salt, thus preventing the entrance of the latter into the protoplasm, has been made by several investigators,¹⁰ and lately

⁹ OSTERHOUT's observations on decrease in the electrical conductivity of fronds of the common kelp (*Laminaria*) under the influence of anæsthetics indicate that the plasma membranes undergo decrease in their general permeability during anæsthesia. Membranes so altered in irritable tissues would presumably undergo the marked permeability increase essential to stimulation — an increase sufficient for loss of semi-permeability, as shown by the conditions in *Mimosa* — with less readiness than normally. If a change in the electrical polarization of the membrane sufficient to cause this increase of permeability in the normal membrane no longer produced such an effect, the tissue would show itself inexcitable to stimulation. My own observations in *Arenicola* larvæ led me to infer several years ago that anæsthetic action was associated with a decrease of permeability. Cf. this Journal, 1909, xxiv, p. 30 *seq.*

¹⁰ The view that pure solutions of sodium chloride exert toxic action by abnormally increasing the permeability of the cell membranes, and that calcium and other antitoxic salts act by counteracting this effect, was first put forward by HERBST, in an extensive paper published early in 1904 (*Archiv für Entwicklungsmechanik*, xvii, p. 306). He describes experiments with young eels, similar to J. LOEB's earlier experiments with *Fundulus* (this Journal, 1900, iii, p. 331), and concludes that the injurious action of the pure NaCl solution on these animals is due largely to a permeability-increasing action on the respiratory epithelium of the gills, permitting undue entrance of the toxic salt; associated with this effect is a loosening of the union between the epithelial cells; both effects depend on an alteration of the surface membranes of the cells, and are prevented by the presence of calcium (*cf.* pp. 439-444). He regards other cases of antitoxic action of salts as probably an expression of similar surface changes (*cf.* also the section on the rôle of calcium, p. 476). G. N. STEWART in 1904 (*American yearbook of medicine and surgery, Medicine*, 1904, p. 527), at the end of a review of the important paper of LOEB and GIES on the antitoxic action of salts (*Archiv für die gesammte Physiologie*, 1902, xciii, p. 246) — in which the effects are referred to changes in the protoplasmic colloids, particularly the lipoids — writes: "The possibility ought to be taken into account that an apparent antitoxic action may sometimes, or in some part, be due to the production of a change in the permeabil-

considerable evidence has accumulated in its favor. The presumption until recently has always been that the toxic substance must first enter the cell protoplasm, and confusion has been introduced by a failure to take into account the properties of the plasma membranes. It has seemed necessary to many, in order to explain the physiological action of salts, to assume that these substances readily enter all cells; and this point of view was in fact definitely upheld by Loeb¹¹ and others, in opposition to Overton's contention that the plasma membrane, as an essentially semi-permeable structure, is in its unaltered state impermeable to neutral salts. If salts do not enter cells, how can they exert toxic or other physiological action? Overton¹² suggested that salts might affect intracellular processes by a purely superficial action, without entrance into the cell, but this point of view has hitherto received little attention.

Observations on *Arenicola* larvæ and sea-urchin eggs led me some years ago to the conclusion that what was essential in the toxic action of pure sodium chloride and similar solutions was *not the entrance of the salt into the cells*, but its general disintegrative or permeability-

ity of the cells to the toxic ions and not to an actual neutralization of their effects within the cell protoplasm." This suggestion has an evident relation to his earlier expressed view that toxic agents like hæmolysins "act primarily by altering the permeability of the cell envelope" (*cf.* this Journal, 1903, ix, p. 80); inferentially, therefore, an antitoxic agent prevents this action. MATHEWS' experiments with *Fundulus* eggs in the summer of 1904 led him to conclude that changes in the permeability of the egg membranes played an essential part in the antitoxic action; thus the coagulative action of salts of cobalt, nickel, and manganese on the egg protoplasm was delayed in the presence of calcium. "We may conclude, therefore, that when calcium is present the cobalt, nickel, and manganese do not enter the egg, and accordingly the embryo is protected from them" (this Journal, 1905, xii, p. 441). Somewhat later LOEB also called attention to the possibility that salts like calcium chloride may prevent the toxic action of sodium chloride solution by altering the surface layer of the protoplasm, thus preventing the entrance of a toxic excess of the salt (*Archiv für die gesammte Physiologie*, 1905, cvii, p. 252), but without bringing forward specific evidence in support of this hypothesis. Recently he has reaffirmed this view, and his latest contribution to the subject (*Science*, N. S., 1912, xxxvi, p. 639) contains definite experimental evidence in its favor.

¹¹ Cf. J. LOEB: Dynamics of living matter (Macmillans, 1906, p. 41 *seq.*), and the article "Ueber physiologische Ionenwirkungen" in OPPENHEIMER'S *Handbuch der Biochemie*, 1909, ii (1), p. 104.

¹² OVERTON: *Archiv für die gesammte Physiologie*, 1904, cv, p. 176.

increasing action on the plasma membranes.¹³ Prevention of this effect, as by the addition of calcium, thus involves antitoxic action. Various experiments were then cited supporting this contention. It was found, for instance, that if *Arenicola* larvæ are first treated for a few minutes with magnesium chloride the permeability-increasing action of subsequent exposure to pure sodium chloride solutions is prevented, and simultaneously the toxic action is greatly reduced.¹⁴ Alteration of surface permeability appears thus to be the essential in this form of toxic action. In general we must assign to the plasma membrane the significance of a diffusion-preventing and hence semi-permeable surface film, whose integrity is essential to the preservation of the normal composition of the protoplasm.¹⁵ Protection against the destruction of this semi-permeability by toxic agents is thus equivalent to antitoxic action.¹⁶ According to this point of view the semi-permeability of the plasma membrane is no merely incidental property, but one essential to the maintenance of the normal composition and properties of the cell; hence agencies or conditions that deprive the membranes of their semi-permeability are toxic, and in such cases the antitoxic agent works by counteracting this action.

Structures like membranes or cilia may be protected against breakdown in pure sodium chloride solution either by salts, as calcium or magnesium chloride, which presumably affect the total colloids of the membrane, or by organic anæsthetics, which act selectively on the lipoids. Antagonisms of this kind are thus not confined to antago-

¹³ Cf. my two papers in this Journal, 1909, xxiv, pp. 14 and 459; also 1910, xxvi, p. 106.

¹⁴ *Loc. cit.*, 1909; cf., e. g., p. 487; cf. also this Journal, 1911, xxvii, p. 296.

¹⁵ For a fuller discussion of this subject cf. Biological bulletin, 1909, xvii, p. 188; this Journal 1910, xxvi, p. 106. MACALLUM (Journal of the Royal Society of Canada, 3d Series, 1908, p. 145), ZANGGER (*Ergebnisse der Physiologie*, 1908, vii, p. 138 and elsewhere), and HOEBER (*Physikalische Chemie der Zelle und der Gewebe*, 3d ed, 1911, p. 252) have assigned a similar significance to the semi-permeability of the plasma membrane. Similar considerations apply to the membranes or other colloidal partitions inside the cell; these may thus play an important rôle in intracellular differentiation, as first suggested by HOFMEISTER in his "Chemische Organization der Zelle," Jena, 1901.

¹⁶ This interpretation of the antitoxic action of salts has been adopted by J. LOEB in his more recent discussions of this problem. Cf. *Science*, N. S., 1911, xxxiv, p. 653; *Biochemische Zeitschrift*, 1911, xxxvi, p. 277; 1912, xxxix, p. 194; xliii, p. 181.

nisms between ions, and the conception of equilibrated salt solutions, valuable as it is, is not broad enough to include the entire range of phenomena.¹⁷ The character and concentration of the ions in the protoplasm and medium are not the only factors determining the condition of the plasma membrane; the non-electrolytes, especially those which influence the state of the lipoids, are perhaps equally important. The above observations furnish direct evidence that the condition of the lipoids in the plasma membranes determines the rate and degree of action of toxic substances as well as of stimulating agencies. These phenomena have an evident relation to those cases where the addition of a lipoid to the medium influences toxic action — whether as activator or inhibitor; cholesterol in particular shows well-marked protective action in a variety of relations;¹⁸ it may protect blood corpuscles against hæmolytic agents; it also has been shown to increase the resistance of artificial lipoid-impregnated membranes to alteration by similar agents.¹⁹ Apparently changes in the state of the lipoids already present in the membranes may have similar effects.

The precise rôle of the lipoids in the plasma membranes requires further research for its elucidation. Their condition in some way exerts a far-reaching control over cell processes. A relation to oxidations has been suggested by some investigators. According to Walde-mar Koch²⁰ it is possible that “phosphatides may, by means of their

¹⁷ LOEB and GIES were unable to obtain satisfactory evidence of antitoxic action between electrolytes and non-electrolytes (*Archiv für die gesammte Physiologie*, 1902, xciii, p. 246), and concluded that non-electrolytes differed fundamentally from electrolytes in their relation to this class of antagonistic action. It would appear, however, that non-electrolytes which influence lipoids form an exception to this rule. The state of these colloids is obviously influenced by other factors than the nature of the ions present.

¹⁸ ZANGGER gives a general discussion of these “antitoxic effects of lipoids especially of cholesterol” in a paper on “die Immunitäts-Reaktionen als physikalisches speziell als Colloid-Phänomen” in *Vierteljahrsschrift der naturforschenden Gesellschaft in Zürich*, 1908, liii, p. 408. Cf. also the article of LANDSTEINER in OPPENHEIMER's *Handbuch der Biochemie*, Bd. ii (1), section on hæmolysis, pp. 444 *seq.*, for a summary of the chief facts, and literature.

¹⁹ PASCUCCI: *Beiträge zur chemischen Physiologie und Pathologie*, 1905, vi, p. 543.

²⁰ KOCH, W.: *Journal of pharmacology and experimental therapeutics*, 1910, ii, p. 265.

unsaturated acids, play a rôle in oxidations or in the attraction of tissues for oxygen." Certain facts of antitoxic action show a certain harmony with this view. Loeb has recently shown that some forms of toxic action are more rapid in the presence of oxygen than in its absence or in presence of cyanide. Apparently oxidation processes are contributory or accessory to the destructive action in such cases.²¹ If for these destructive oxidations the participation of the lipoids in the membranes is necessary, checking these oxidations might prevent the toxic action by preventing alteration of the lipoids, thus preventing breakdown of the membrane. Similar considerations would, however, apply to any kind of toxic action in which oxidations were a factor. Loeb's papers on this subject contain no evidence that he regards the membranes as the seat of the essential toxic action. Cyanides do, however, check the permeability-increasing action of sodium salt solutions on echinoderm eggs, just as do lipid solvents or calcium salts.²² This fact harmonizes with the supposition that oxidation processes play a part in the breakdown of the membranes under certain kinds of toxic action; and possibly the lipoids — since their modification by anæsthetics has a similar protective effect — are concerned in these oxidations. Further discussion of this problem is best deferred until the accumulation of further data.

It must be recognized that the problem of the nature and conditions of toxic and antitoxic action — whether of salts or of other substances — falls into the same essential category with the wider problem of the nature and conditions of physiological antagonisms in general. The relations normally existing in organisms between stimulation and inhibition — which are evidently opposite phases of the same process — call for consideration here. According to the evidence of the above experiments, stimulation and inhibition under the influence of salts and anæsthetics are — like the toxic and antitoxic action of these substances — dependent on changes in the state of the limiting membranes. I have already discussed the conditions of stimulation in some detail. The conditions of the inverse process of inhibition in the normal organism are probably fundamentally similar to those of artificial inhibition under the influence of anæsthetics, salts, or the electric current (anelectrotonus). On this view

²¹ LOEB, J.: *Biochemische Zeitschrift*, 1910, xxvii, p. 304; xxix, p. 80.

²² LILLIE, R.: *this Journal*, 1912, xxx, p. 1.

inhibition is the expression of a change in the state of the plasma membranes, opposite in kind to that associated with stimulation; *i. e.*, the membrane is rendered by inhibition less susceptible than normally to permeability increase and hence to stimulation. Exactly how this occurs is as yet unknown. If, however, stimulation is the expression of a change of electrical polarization in the one direction (depolarization), a polarization change in the opposite direction is probably concerned in inhibition. In the reflexes of higher animals excitation of one set of motor neurones involves the inhibition of the antagonistic set, as Sherrington has shown, though the conditions of this interference remain unexplained. *An electrotonic influence of one set of neurones on the other* seems the most likely basis of such an effect; and this interpretation is supported by certain phenomena of galvanotropism in which the orienting or directive influence of the external electric current depends on a simultaneous inhibition and reinforcement of opposite motor innervations which are undoubtedly of central origin.²³ Sherrington has put forward the hypothesis that alterations in the state of the membranes at the synaptic junctions are a condition of inhibition,²⁴ an explanation plainly consistent with the general point of view advocated in this

²³ Cf. the observations of LOEB and GARREY on *Amblystoma* (Archiv für die gesammte Physiologie, 1896, lxxv, p. 41), and LOEB and MAXWELL (*Ibid.*, 1896, lxxiii, p. 121) on *Palæmonetes*.

That is, external electrical conditions that favor the activity of one set of neurones inhibit the activity of the antagonistic set. Similarly, the electrical variations that *normally* accompany the activity of, *e. g.*, the flexor neurones may be incompatible with — or, in other words, may inhibit — the activity of the extensor neurones. Neurones so related would be capable of alternating but not simultaneous activity. I venture this suggestion here as possibly throwing light on the mechanism of a very obscure process. From the above observations LOEB concluded “that the nervous elements of the flexors and extensors in the central nervous system which are affected by the ions (in galvanotropism) possess an opposite orientation,” such that an electrical current which produces catelectrotonus (or excitation) in one causes anelectrotonus (or inhibition) in the other. Cf. his Comparative physiology of the brain, p. 162, and the discussion following, also the above papers. He does not, however, so far as I am aware, consider the case of reciprocal inhibition. The structural conditions in the central nervous system may quite possibly be such that the action current of one neurone instead of stimulating *inhibits* the activity of the adjoining antagonistic neurone.

²⁴ SHERRINGTON: Integrative action of nervous system, p. 192.

paper. The conditions of mechanical inhibition in the ctenophore swimming plate suggest that the membrane of the inhibited tissue becomes increasingly resistant to permeability change,²⁵ and in the case of cells like motor neurones the conditions are probably essentially similar. It is highly desirable to reach, if possible, a valid unitary point of view from which to consider these and related phenomena; its attainment would greatly facilitate effective and well-directed research; and the indications are already strong that the conception of the plasma membrane as a structure whose condition exerts a controlling influence over cell processes — largely through changes of electrical polarization conditioned by changes of permeability — will furnish this point of view.

SUMMARY.

1. In the action of pure isotonic NaCl solutions on *Arenicola* larvæ the most evident effects are: (1) strong stimulation of the musculature, causing intense and prolonged contraction, (2) increase in the permeability of the pigment-cell membranes sufficient to allow visible exit of pigment, (3) immediate arrest of ciliary movement, followed by disintegration of the cilia, and (4) a general toxic action.

2. In the presence of a large number of anæsthetics, in concentrations corresponding to those producing typical neuromuscular anæsthesia in sea water, all of these characteristic immediate effects of the pure salt solution are diminished or prevented.

3. In general, the permeability-increasing action and the stimulating action of the salt solution undergo closely parallel decrease or prevention in the presence of the anæsthetic. Prevention of sudden permeability increase thus seems equivalent to prevention of stimulation; it is also equivalent to prevention of the immediate toxic action of the solution. The anti-stimulating and the anti-cytolytic effects of the anæsthetic thus show a definite parallelism.

4. The conclusion is drawn that in anæsthesia the essential effect is a temporary alteration in the condition of the surface films or plasma membranes of the irritable elements, of such a kind that these membranes no longer undergo, under the usual conditions of stimulation, the rapid increase of permeability essential to this process.

²⁵ LILLIE, R.: this Journal, 1908, **xxi**, p. 200; cf. p. 215 *seq.*

5. The membranes thus become during anæsthesia increasingly resistant to permeability-increasing agencies: this involves increased resistance to those forms of toxic action which depend on destruction of the normal semi-permeability of the membranes. Hence the association of an anti-cytolytic or antitoxic action with the anti-stimulating action of the anæsthetic. These observations also indicate that the degree of resistance of the membranes, and of other colloidal structures like cilia, is intimately dependent on the state of their component lipoids.

THE FORMATION OF INDOPHENOL AT THE NUCLEAR AND PLASMA MEMBRANES OF FROGS' BLOOD CORPUSCLES AND ITS ACCELERATION BY INDUCTION SHOCKS.

PLATE I.

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In my earlier paper on the localization of the formation of colored oxidation products in the cells and tissues of the frog,¹ I described experiments in which dimethyl-*p*-diamino-benzene $C_6H_4.NH_2.N(CH_3)_2$ was used (instead of the usual unsubstituted compound $C_6H_4(NH_2)_2$) in conjunction with α -naphthol for the intracellular production of indophenol. A mixture of these two substances forms on oxidation dimethyl indophenol, a deep blue, water-insoluble compound which is deposited within the cells in the form of conspicuous granules; these show in many cases a highly definite distribution; thus in red corpuscles and leucocytes a tendency to deposition at the surface of the nuclear membrane is very constant and pronounced. This appearance is so definite that it seemed at that time to afford strong support to the view that the cell nuclei have a special relation to oxidation processes. The photographs on plate I (figs. 1 and 2) illustrate this condition in corpuscles that have lain for some minutes in a solution of α -naphthol and dimethyl-*p*-diamino-benzene in physiological salt solution. It will be seen on referring to these photographs that the granules at the boundary of nucleus and cytoplasm in the erythrocytes are larger and more densely massed than elsewhere in the cell.

There is little doubt that the regions where the indophenol is

¹ *Amer. Journ. of Physiol.*, vii, p. 412, 1901.

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most densely deposited within these cells correspond to the regions of its most rapid formation, and hence to the regions of most active oxidations. Indophenol granules show an active Brownian movement when freely suspended,—as in a solution of α -naphthol and p -diamino-benzene undergoing spontaneous oxidation; but when the granules are deposited in the interior of the cells little or no movement is perceptible, and under these conditions they must tend to remain in the place of their formation. Direct observation shows that their aggregation at the nuclear membrane of erythrocytes is not the effect of a secondary gathering in this situation, subsequent to formation elsewhere in the cell; the first granules that make their appearance in erythrocytes after placing in the indophenol-forming solution almost always occupy this position, and it is not until later that granules appear elsewhere in the cell. The region adjoining the nuclear surface in erythrocytes appears thus to be a region of relatively rapid oxidation. Evidence that oxidative processes of this kind may show definite localization in cells acquires especial significance in view of Vernon's recent observations, according to which the rate of formation of indophenol is a fair index of the general oxidative power of the tissue;² if this is the case, the conditions of formation of this compound in cells probably correspond in the main to those of the other and physiologically important oxidations.

Further study has however convinced me that the nuclear surface is not, in general, necessarily the seat of more active oxidations than the other free surfaces or phase boundaries in cells. The oxidation process appears to be strongly influenced by the conditions at surfaces; adsorption and variations of phase-boundary potentials are probably the chief factors in this influence, as apparently in other forms of catalysis due to surface action. I find that in voluntary muscle cells the indophenol formation shows no evident relation to the scattered superficial nuclei, but appears diffused throughout the cytoplasm. Again, in frozen sections of the frog's brain and spinal cord the distribution of most active indophenol formation does not correspond to the distribution of nuclei in these tissues; the oxidation is, in fact, more active in the white than in the gray matter. These facts,

² Vernon: *Journ. of Physiol.*, xlii, p. 402, 1911.

as well as various more general considerations partly discussed below, suggest that the formation of indophenol at the nuclear surface in blood corpuscles is not to be regarded as indicating a specific relation of nuclei to oxidations; it seems more probable that it indicates the existence of a general relation of the cell-surfaces or protoplasmic phase boundaries to oxidations. There are other facts indicating this. Warburg's observations on sea-urchin eggs show that alkali may modify oxidations without penetrating the cell—apparently by altering the state of the general cell-surface or plasma membrane.³ Nernst's theory of electrical stimulation implies that the rate of oxidation in irritable tissues is increased by varying the state of electrical polarization of the semi-permeable cell surfaces; *i.e.*, increase in oxidation is a very general consequence of electrical stimulation, whose essential condition is alteration of the electrical polarization at such surfaces.

Re-examination of the conditions of indophenol formation in frog's blood corpuscles has confirmed my former observations of a relatively rapid oxidation at the nuclear surface; the appearances indicate also that a second region of relatively rapid oxidation exists near the general outer surface of the cell, or just within the plasma membrane. It is difficult to obtain unequivocal proof of this in the case of erythrocytes, on account of the flattened shape of these cells; but when the cells are slightly crenated and are viewed in face with a high power, the granules of indophenol often show a network-like disposition which apparently corresponds to the folds of the surface; examination with the oil-immersion lens—especially when the cells are viewed edgewise—also often shows a denser distribution of granules just within the cell-surface than in the region intermediate between plasma and nuclear membranes. The region of densest deposition of granules in erythrocytes is, however, always the nuclear surface, and apparently this is the region of most active oxidation in these cells.⁴ In leucocytes a relation of oxidation to the general

³ Warburg: *Zeitschr. f. physiol. Chem.*, lxvi, p. 305, 1910.

⁴ Warburg's observations on the rate of oxygen consumption by the red corpuscles of birds show an interesting parallel with those just cited (cf. Warburg: *Zeitschr. f. physiol. Chem.*, lxx, p. 413, 1911; *Munch. med. Wochenschr.*, lviii, p. 289, 1911). These cells show a much higher oxygen consumption, especially when newly formed, than the non-nucleated ery-

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cell-surface is more distinct. Indophenol is formed in these cells much more rapidly than in erythrocytes, and in a mixture of α -naphthol and dimethyl-*p*-diamino-benzene solutions, of the composition given below (page 243), the surface layer of the cytoplasm of large leucocytes, as well as the region adjoining the nucleus, quickly becomes filled with large and conspicuous granules. The presence of numerous granules just within the cell-surface is highly characteristic of leucocytes that have lain for a few minutes in the indophenol-forming solution. Later the whole cytoplasm becomes densely laden and the original inequality of distribution is obscured. In some of the photographs reproduced with this article rows of granules just within the cell-surface are clearly seen (see figs. 4 and 5).

These observations show that a close relation exists between oxidations and certain intracellular surfaces or phase boundaries. It is theoretically improbable that the surfaces of semi-permeable membranes like nuclear or plasma membranes are alone concerned in these processes. The surfaces of other colloidal structures probably play a similar part in cells; thus in muscle cells it is possible that the large surface of contact between fibrils and sarcoplasm forms a region active in oxidations, although there is no direct evidence of this as yet; if so, the high oxidative activity of these cells may in part be accounted for. Increased reaction velocity in polyphasic systems with large surface extent is a frequently observed phenomenon; the catalytic action of colloidal metals has been explained by Bredig and others as an

throcytes of mammals. Alternate freezing and thawing destroys the cytoplasm, but leaves the nuclei intact; cells thus altered show an unimpaired or even increased rate of oxidation. Evidently the general cell-surface is not essential to oxidation in these cells; if however the *nuclear* surface is the active region in birds' erythrocytes, as in those of frogs, Warburg's results may readily be accounted for. His further observation that salts like calcium, magnesium, and barium chloride influence oxidations *after*, but not before, destruction of the plasma membranes also receives a consistent explanation, since the plasma membranes are normally impermeable to these salts, which accordingly have free access to the nuclear membrane only after the plasma membranes are destroyed. On the other hand, phenyl, methyl, and ethyl urethanes, and hydrocyanic acid, which penetrate the plasma membranes with ease, influence oxidations equally in intact and injured erythrocytes.

instance of such surface action, and this action is very generally referred to increased concentration of the interacting substances at the phase boundaries. It is probable that the electrical condition of these surfaces also constitutes an important factor in the catalytic action.⁵

The importance of surface processes in cell activities has been widely recognized of recent years, especially since the rise of colloidal chemistry and its application to physiological problems. The character of the electrical polarization at surfaces is known to influence profoundly the adsorptions at such surfaces,⁶ and any chemical changes dependent on adsorption (as catalyses of the class referred to above) must be correspondingly influenced by the electrical condition of the surfaces. Hence chemical processes occurring in polyphasic systems must in many cases be influenced by changes in the polarization of the surfaces, to a degree which under some conditions may largely determine the general course and velocity of certain reactions; and the possibility that the chemical effects of stimulation may belong in this category thus requires consideration. According to Nernst's theory, changes in the electrical polarization of the plasma membranes form the essential condition of electrical stimulation. The same is almost certainly true of other forms of stimulation, including the normal, since all are accompanied by electrical variations whose characteristics point unmistakably to variations of phase-boundary potentials as their determining condition.⁷ The rate of oxidation in voluntary muscle cells is increased

⁵ See the communication of S. W. Young: On the Influence of Light on the Electric Potential of Bacterial and Other Suspensions, in the *Proc. of the Soc. for Exp. Biol. and Med.*, x, p. 151, 1913.

⁶ Cf. L. Michaëlis in Koranyi and Richter's *Physikalische Chemie und Medizin*, ii, 1906, pp. 347 seq.; Michaëlis and Ehrenreich; *Biochem. Zeitschr.*, x, p. 283, 1908; Höber: *Physikalische Chemie der Zelle und der Gewebe*, 1911, p. 294; Wolfgang Ostwald: Die neuere Entwicklung der Kolloidchemie in *Kolloidchemische Beihefte*, 1912, iv, p. 16. Ostwald distinguishes "electrical adsorption" from adsorption due to lowering of the surface tension at the phase boundary. In general adsorption occurs whenever the energy potential at the phase boundary is lowered by the accumulation of the dissolved substance in this region.

⁷ Cf. my paper on the relation of membrane-changes to stimulation and conduction, in *Amer. Journ. of Physiol.*, xxviii, p. 197, 1911.

many times by stimulation; and investigation of the action current of this tissue with the thread galvanometer shows that stimulation is normally associated with a rhythmical electrical variation of a definite rate; this rate varies with temperature according to the chemical temperature coefficient,⁸ *i.e.*, shows a general parallelism with the oxidative and other chemical activity of the tissue as influenced by temperature change. An interdependence of some kind is thus indicated, and it seems likely that this interdependence is direct, and that the polarization changes form the immediate condition of the oxidations. The mechanism of intracellular oxidations is still largely obscure, and it is admitted by most biological chemists that the known properties of oxidases cannot account for the character and high velocity of the intracellular oxidations. No enzymes or combinations of enzymes and co-enzymes can accelerate the oxidation of sugar or lactic acid to anything like the degree required. Such facts suggest that the oxidases are not the main factors in intracellular oxidations, but play a merely accessory part; their presence may be favorable to rapid oxidation (by diminishing resistance), but the essential determining conditions appear to be of a quite different kind. Some feature or features of the structural organization of the cell must be fundamentally concerned, since destroying cell structure always greatly diminishes the oxidative activity of the tissue.⁹ Now the semi-permeable membranes of irritable tissues constitute the structural elements which are primarily essential to electrical stimulation, and hence to the increase of oxidation which is associated with stimulation. According to Nernst's theory, stimulation involves changes in the electrical polarization of these membranes. Such changes of surface polarization must involve corresponding changes in the polarization of the other polarized surfaces within the cell; *i.e.*, the conditions of equilibrium of the double layers at these intracellular surfaces will be altered by altering the polarization of the general cell-surface, and their state of polarization will undergo corresponding change; hence the normal variations of polarization at the cell-surface accompanying stimulation must involve similar changes at the active surfaces throughout the cell, and it is these polarization changes

⁸ Cf. Piper: *Arch. f. (Anat. u.) Physiol.*, 1910, p. 207.

⁹ Cf. Fletcher and Hopkins: *Journ. of Physiol.*, xxxv, p. 287, 1907.

—according to the hypothesis which I am presenting—that mainly determine the increase in the rate of oxidation. Similar or related views have been tentatively put forward by a number of investigators,¹⁰ but hitherto little or no experimental evidence in their support has been adduced. The experiments about to be described show that the formation of indophenol in leucocytes—a process largely dependent on surface action as already described—can in fact be markedly accelerated by the passage of induction shocks through a suspension of corpuscles on a slide.

EXPERIMENTAL.

In the following experiments the blood corpuscles of the frog have been used. Immediately after shedding, the blood is mixed with a solution consisting of nine volumes $\frac{M}{8}$ NaCl *plus* one volume $\frac{M}{8}$ potassium oxalate; this solution prevents clotting and breakdown of leucocytes. The suspension of corpuscles is then mixed with the solution of the indophenol-forming reagents in slightly alkaline physiological salt solution. The solution which I have chiefly used is made (shortly before using) by mixing a saturated solution of α -naphthol in alkaline isotonic NaCl solution ($\frac{M}{8}$ NaCl containing $\frac{M}{100}$ Na_2CO_3) with a 0.5 per cent to 1 per cent solution of Merck's dimethyl-*p*-diamino-benzene in $\frac{M}{8}$ NaCl. These concentrations are favorable to the rapid formation of indophenol within the cell. With more dilute solutions the formation of indophenol is slower and its acceleration by induction shocks is less evident. Usually equal volumes of these solutions (1 cc. of each) are mixed in a test tube, and the resulting solution is then mixed intimately with a few drops of the suspension of corpuscles either in a watch glass or on a slide. This preparation may be mounted and examined at once or after the desired interval of time has elapsed. Leucocytes left in this solution (with sufficient access of oxygen) become deeply laden with indophenol granules in a few minutes. In erythrocytes the deposition of granules is more gradual; in these cells the first formed granules typically appear at the nuclear surface, as already described. Figures 1 and 2 are photographs of corpuscles taken after remaining for twenty-five and fifty-two minutes respectively

¹⁰ Cf. Warburg, 1910, *loc. cit.*

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in a solution of the above composition. By this time the leucocytes are so densely laden with indophenol as to prevent the appearance of mere clumps of granules. The erythrocytes are much less deeply impregnated and the contours of the nuclei are plainly marked by the deposition of granules.

The accelerating influence of induction shocks on this oxidation may be demonstrated as follows. Immediately after mixing with the reagent, as above described, a few drops of the suspension of blood corpuscles are mounted on a specially prepared slide crossed by two fine parallel platinum wires about 2 cm. apart. The slide is already in position on the microscope stage; the wires are connected to the secondary coil of an inducturium (Porter's model) arranged for "tetanizing" currents, and a succession of shocks is passed through the preparation. If a suspension of corpuscles so treated is compared with a control consisting of a portion of the same suspension similarly mounted on a precisely similar slide (so as to have conditions of oxygen supply, etc., as nearly alike as possible), but not subjected to induction shocks, a marked difference soon becomes evident. Within a brief period, varying according to the strength of the shocks and concentration of the solution, the leucocytes in the "stimulated" preparation are seen to be plainly more deeply impregnated with indophenol than those of the control. The contrast is very striking if the conditions of concentration, number of corpuscles,¹¹ and strength of stimulation are favorable (see plate I, figs. 3-5). The difference is not due to the oxygen liberated at the wires serving as electrodes, for the acceleration of indophenol formation is seen throughout the entire region between the electrodes.

The following protocols of experiments will illustrate the character of these observations.

November 4, 1911. The blood of a large frog was mixed with an approximately equal volume of oxalate-containing sodium chloride solution. A drop of this suspension of corpuscles was placed on a slide provided with platinum wires; a drop of the indophenol-forming mixture was placed in

¹¹ Too dense a suspension of corpuscles is unfavorable to this demonstration, probably because the oxygen is too rapidly abstracted by the erythrocytes, leaving less available for oxidation in the leucocytes.

contact with the drop of suspension and a cover glass was put in place. Induction shocks were then passed.

In six successive experiments of this kind the leucocytes were found to form indophenol granules more rapidly under this treatment than in the untreated control. In some experiments the contrast at the end of two or three minutes was very striking; the leucocytes near the edge of the cover glass, where most oxygen was available, being then so deeply laden as to appear like masses of granules, while in the control only a moderate deposition of granules had taken place.

Numerous other experiments showed similar results. Usually the suspension of corpuscles and the indophenol-forming mixture were mixed before mounting. The following typical experiments are cited in further illustration.

November 15, 1911. The suspension of corpuscles was mixed with the freshly prepared indophenol-forming solution in a watch-glass and then mounted on the slides with platinum wires. Induction shocks from a Porter's inductorium with three Edison primary cells and coils 3 cm. apart were passed through the preparation. Nine experiments were performed under these conditions. The following results are typical.

EXPERIMENT 3. At 12.11 the current is started through the preparation. The granules appear rapidly in the leucocytes; at 12.16 the field of the stimulated preparation is full of intensely colored leucocytes; in the control, although many leucocytes are well laden with indophenol, the number of deeply impregnated cells and the degree of loading are much less. By 12.20 the erythrocytes of the stimulated preparation also show a distinctly deeper tint than those of the control.

EXPERIMENT 4. Current started at 12.37. The result is similar to the above; in three minutes there is a decided contrast between experiment and control. At 12.43 the erythrocytes also show considerable indophenol; the nuclear membranes appear to be impregnated with the dye, and stand out sharply. Some leucocytes show granules at the nuclear surface, and others show rows of granules just within the cell-surface.

EXPERIMENT 8. Conditions and results the same. The contrast between the microscopic fields of experiment and control under a low power is striking; eight minutes after starting the current the field of the stimulated preparation appears dotted at intervals with intensely blue-black dots corresponding to the leucocytes; in the control the leucocytes stand out much less conspicuously.

The above records are sufficient to indicate the general character of these observations. It will be noted that the induction shocks used in the above experiments are of considerable strength. Weaker shocks also accelerate the rate of oxidation in leucocytes but less obviously. It is also noteworthy that the effect on the

erythrocytes is comparatively slight; the oxidative activity of these cells, besides being much less energetic than that of the leucocytes, is less easily influenced by electrical stimulation, although after passing strong shocks for some minutes there is an appreciable increase in the formation of indophenol. The much higher oxidation rate and greater responsiveness of the leucocytes correspond to the more active part which these cells play in the organism; thus it is not surprising to find that their oxidation rate—and hence their general rate of metabolism and output of energy—is more easily altered by changes of condition. Leucocytes, although relatively inert as compared with (*e.g.*) muscle cells, have in fact many of the characteristics of irritable cells. Hamburger has shown that their phagocytic activity may be increased artificially by a variety of substances, including calcium salts and lipid solvents in low concentration.¹² The conditions under which oxidations are accelerated in these cells are thus probably of the same essential kind as those prevailing in irritable cells in general.

The possibility naturally suggests itself that various chemical processes other than oxidations may be similarly influenced by changes of polarization at the intracellular surfaces. The formation of indophenol is in fact a synthesis involving dehydration, as well as an oxidation, but probably no especial significance attaches to this fact. What seems to be significant is that the chemical process occurs in close relation to the intracellular surfaces, and is influenced by changes in the electrical polarization of these surfaces.¹³ It seems likely that conditions of this kind play an important general rôle in cell metabolism. The precise conditions of the above electrochemical effect are largely obscure to me at present. The analogy with photochemical action seems

¹² Hamburger and Hekma: *Biochem. Zeitschr.*, ix, p. 275, 1908; Hamburger, de Haan and Bubanovic: *Archives Néerlandaises des Sciences Exactes et Naturelles*, Ser. III, B., i, p. 1, 1911. Hamburger's recent book: *Physikalisch-chemische Untersuchungen über Phagozyten*, Wiesbaden, J. F. Bergmann, 1912, contains a full account of these and related observations.

¹³ The formation of peroxides may possibly be favored by this process, just as the formation of ozone and hydrogen peroxide is favored by the passage of electric discharges through moist air. There is good evidence that peroxides play an important—though it may be a supplementary—rôle in protoplasmic oxidations.

perhaps the most illuminating. In both cases increased displacement, or increased range of movement, of electrons—due to the electro-magnetic effect of the ether waves, or of the variation of surface polarization—and consequent facilitation of the electron transfers that condition the chemical change, are probably the fundamental factors of the action. Further discussion of this matter would, however, be inappropriate in this place.

SUMMARY.

1. In frogs' blood corpuscles the formation of indophenol by the intracellular oxidation of a mixture of α -naphthol and dimethyl-para-diamino-benzene takes place most rapidly in the immediate neighborhood of the nuclear and plasma membranes. The conditions at the surfaces of these structures are thus particularly favorable to rapid oxidations.

2. The passage of induction shocks through a suspension of corpuscles in the indophenol-forming solution accelerates this oxidation; this effect is slight in erythrocytes and well marked in leucocytes.

3. These observations indicate, in general, (a) that the intracellular surfaces or phase boundaries play an active rôle in oxidations in living cells, and (b) that variations in the electrical polarization of these surfaces form an important factor in these oxidations, and especially in the increase of oxidations following stimulation.

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EXPLANATION OF PLATE.

The photographs were made by Dr. H. G. Kribs, of the Zoölogical Department, University of Pennsylvania, to whom I take pleasure in expressing my best thanks. The magnification is about 500 diameters. The exposures were brief (5 to 10 seconds) and were made within one to two minutes after the stimulating current had ceased. The corpuscles shown in these photographs are entirely typical of the conditions throughout the preparation.

Fig. 1. Showing spontaneous formation of indophenol in corpuscles. The cells were photographed after lying undisturbed for twenty-five minutes in the indophenol-forming solution. The densely laden central corpuscle is a leucocyte. The round dots scattered over the field are indophenol granules; the halo about each is due to Brownian movement.

Fig. 2. Corpuscles from a similar "unstimulated" preparation after fifty-two minutes in the solution. The round black mass just above the middle of the figure is a leucocyte. The rows of granules at the nuclear surface in the erythrocytes are typical.

Fig. 3. Stimulated preparation. The corpuscles were placed in the freshly prepared indophenol-forming solution and one-half minute later induction shocks were passed for three minutes through the preparation (mounted on a slide as described in the text. Two Edison primary batteries; Porter's inductorium with coils 2 cm. apart). Exposure was made one minute after current ceased. Four leucocytes are shown in the field; the one at the left is less densely laden than the other three.

Fig. 3a. Control of figure 3. The corpuscles were mounted as in the experiment of figure 3 but not subjected to induction shocks. Exposure was made after remaining for the same length of time (five and one-half minutes) in the solution. Six leucocytes in the field, all showing considerable formation of indophenol, but much less than in the stimulated corpuscles.

Fig. 4. Conditions the same as in the experiment of figure 3. Three deeply laden leucocytes in the field.

Fig. 4a. Control of figure 4 (in the solution for the same length of time, but unstimulated). Two leucocytes seen, showing much less indophenol formation than those of figure 4.

Fig. 5. Conditions like those of the preceding experiments, but shocks passed for only two minutes. Exposure four and one-half minutes after placing in the indophenol-forming solution. Two leucocytes are seen, showing typical conditions.

Fig. 5a. Control of figure 5. In solution for the same length of time before exposure. Two typical leucocytes shown.

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THE PHYSICO-CHEMICAL CONDITIONS OF ANESTHETIC ACTION¹

UNDER certain well-defined artificial conditions, as well as under some that are normal, the living system—organism, tissue or cell—becomes temporarily inactive and irresponsive to stimuli. When such an artificially induced state of inhibition is well marked and lasting it is called anesthesia, or in a somewhat more restricted sense, narcosis. This condition may last for hours or even days, but apparently not indefinitely; and when it passes off the normal vital activities and properties return unimpaired. This apparently complete *reversibility* is one of the most remarkable features of anesthesia, and distinguishes it from death—a perhaps related but characteristically irreversible change. The terms “anesthesia” and “narcosis” are somewhat differently applied, although they have the same essential significance; the former relates to any temporarily insensitive condition, however produced, while “narcosis” usually means an anesthesia produced by chemical substances. I shall use the term anesthesia throughout the present address to designate any temporary or reversible lowering or loss of the normal vital responsiveness, or of the normal automatic vital activity, under the influence of certain artificial substances or conditions. Anesthesia, as thus defined, may be exhibited by the most various organisms and cells, if not by

all. It is fully as characteristic of plant cells as of animal cells, although its manifestations may be less obvious and striking in the former group of organisms. In its most familiar aspect the complete organism, *e. g.*, a man, or an isolated living tissue, as a nerve or muscle, fails during anesthesia to show any response to a stimulus which normally excites it strongly. In other words, the capability of responding to stimuli—what we call “irritability”—is in anesthesia diminished or lost. When the condition passes off the normal responsiveness returns unimpaired. Thus a muscle exposed to ether vapor soon ceases to contract on stimulation; under the same conditions a nerve ceases to conduct; in motile plants like sensitive plants the characteristic osmotic motor mechanisms cease to act. Automatic activities like ameboid movement, ciliary movement, protoplasmic flowing, cell division, and growth may also be brought temporarily to a rest by anesthetics. Claude Bernard showed long ago that seedlings ceased growth in an ether-impregnated atmosphere, and resumed it when the ether was removed. Fertilized egg-cells cease to divide in the presence of an anesthetic in appropriate concentration, although they remain living and proceed with cell-division and development when the anesthetic is removed. Other less evident cell-processes, including metabolism, are similarly affected; the rate of oxidation is

¹Lecture given before the Chemical Society of Washington, April 11, 1913.

usually slowed during anesthesia, though there are exceptions to this rule.

It should be remembered that such decrease of the vital activity or responsiveness is not a solely artificial phenomenon. Conditions physiologically resembling anesthesia occur normally in the life of many organisms; sleep is in fact a kind of physiological regularly recurring narcosis due apparently to accumulation of certain metabolic products in the blood or tissues. Again, all irritable tissues lose their responsiveness for a brief period following excitation; this is the so-called "refractory period," which has been compared with narcosis by some physiologists. The resemblance in this case is probably superficial; but I call attention to this phenomenon in order to show once more that temporary loss of irritability may occur under normal or physiological conditions as well as under artificial. There are also noteworthy resemblances between narcosis and fatigue. Thus the degree of irritability of a tissue may vary within a wide range under normal as well as artificial conditions.

We shall first inquire under what general conditions irritable tissues undergo reversible decrease or loss of irritability. These conditions are various. One of them is cold. The living system operates within a narrow range of temperature. Most irritable tissues or cells become less responsive or lose irritability as the temperature approaches zero. In a muscle or nerve of a cold-blooded animal the ability to respond to stimulation is not necessarily decreased by a moderate reduction of temperature—in fact, slight cooling may increase the irritability of nerve; the tissue responds in the typical manner, but the *rate* of the response—as indicated by the duration of the single contraction in muscle or of the electrical variation in nerve—is always decreased, typically to a degree corresponding to the

usual temperature-coefficient of chemical reaction-velocity. In the neighborhood of zero stronger stimuli becomes necessary to elicit a response, and eventually none may appear. Different organisms vary in these respects. In some animals, as tropical medusæ, irritability is abolished or greatly lowered at a temperature considerably above zero. The same is true of warm-blooded animals. On return to normal temperature irritability is restored.

Another condition producing effects resembling anesthesia is lack of oxygen. This retards or arrests activity in many cases; *e. g.*, the nerve cells of vertebrates are very susceptible to lack of oxygen; nerve trunks, on the other hand, are relatively insusceptible. Cell-division—*e. g.*, in developing egg-cells—usually ceases if the oxygen supply is insufficient. Contractile activities are decreased or abolished. Many organisms, however, show only slight immediate effects; this is true of many Protozoa; Vorticellæ, for instance, remain contractile for some time after simple removal of oxygen from the medium, although they are at once paralyzed by anesthetics. Such facts oppose the view held by Verworn and others, that the anesthetic acts primarily on the oxidative mechanism of the cell. It is true that the rate of oxidations in active tissues is lowered during anesthesia, but this effect is rather a consequence than a cause of the lessened activity. Obviously wherever free oxygen is necessary to the normal activities of a tissue its withdrawal will arrest those activities. But the effects produced by lack of oxygen are not to be identified with anesthesia because of such incidental resemblances.

There are also a number of physical conditions that may deprive a cell temporarily of irritability. Thus mechanical shock may have this effect, which, however, is probably to be regarded as essentially a consequence of over-stimulation, causing abnor-

mal prolongation of the refractory period.² The same is probably true of the insensibility produced by strong electrical currents. Under certain conditions, however, the electric current may produce effects closely resembling typical anesthesia. This occurs when a weak constant current is passed through an irritable tissue like muscle or nerve; during the flow of the current the irritability of the tissue is modified in the neighborhood of the two electrodes, being heightened at the cathode and lowered at the anode; and in this latter region the nerve may become completely insensitive to stimuli that ordinarily cause strong excitation. The inexcitable state thus produced is called "anelectrotonus"; it is in reality a form of local anesthesia, and as such has been employed for the alleviation of pain in sciatica and similar conditions. Muscle is affected in a similar manner; the frog's heart may thus be rendered locally incapable of contraction, as in the simple class-experiment familiar to all physiologists. This action of the current probably depends on its altering the electrical polarization normal to the membranes of the irritable elements—only in a direction the inverse of that causing stimulation.³ There is much evidence that the state of polarization of the semipermeable membranes bounding the irritable elements is an important factor in determining the degree of responsiveness to stimulation; the facts of electrotonus indicate that by altering the polarization by an external current the irritability of the tissue may be changed in the direction either of increase or of decrease.

Irritability may, however, be more read-

² This apparently corresponds to the period of increased permeability and depolarization accompanying stimulation.

³ *I. e.*, reinforcing instead of diminishing the normal or physiological polarization of the membranes.

ily modified by the use of chemical substances than by any other means, and, as is well known, many such substances are in daily use in medical and surgical practise for procuring local or general insensibility to pain—hence the application of the name "anesthetic" to the large class of substances possessing this property. When we inquire into the chemical nature of such substances we find that anesthetic property is confined to no special class, but is exhibited by substances of the most diverse chemical character. Acids in low concentration depress the irritability of many tissues; in some cases alkali has this effect; gases like carbon dioxide and nitrous oxide have marked anesthetic action; solutions of magnesium, calcium and strontium salts cause local anesthesia in frog's muscle and nerve; pure solutions of sugar and other indifferent non-electrolytes have similar effects; in these tissues irritability depends on the presence of certain electrolytes, especially sodium salts, in the media and returns on replacing the tissues in solutions containing these salts. But the most significant relationships are seen in the case of the large class of substances, differing widely in chemical constitution and properties, which possess in common the physical property of dissolving fats or of dissolving in fats. These substances include the majority of the anesthetics in common use, as ether, chloroform, ethyl chloride, urethane, etc. The connection between fat-dissolving power and anesthetic property was in fact early recognized—first by Bibra and Harless in 1847; and this relationship is of great physiological significance, since it indicates that the anesthetic is selective in its action on the cell constituents, and produces its effects by changing the state of fatty or fat-like substances in protoplasm. It indicates further that the state of these substances determines the degree of irritability of the cell. All cells, so

far as known, contain such substances; they are the so-called "lipoids" which include lecithin and cholesterin, and various other ether-soluble compounds of usually complex constitution. Historically these substances were first grouped into a class simply on account of their fat-like solubilities, so that they form, chemically speaking, a somewhat heterogeneous group, some members of which, as cholesterin, are not fats in any sense. Others, as lecithin, are more closely related to the fats proper. They appear to be invariable constituents of protoplasm—a fact which in itself is not surprising in view of the amino-acid constitution of proteins: since proteins are largely derivatives or condensation-complexes of amino-fatty acids, fat-like substances might be expected to appear in cells during metabolism. It is clear, however, that these substances are not mere by-products of protein metabolism, or reserve material like fats, but play a fundamental rôle in cell-processes; the profound physiological effects produced by all lipoid-solvent substances are a sufficient proof of this, although regarding the precise nature of this rôle we know little as yet. In some intimate way the lipoids appear to be essential to the irritability of the cell, and altering their state causes corresponding changes of irritability.

About fifteen years ago Overton and Hans Meyer investigated the relation between the lipoid-solvent power of a large number of organic anesthetics and the intensity of their narcotic action, and reached independently the conclusion that the chief factor determining this action was the value of the partition-ratio of the anesthetic between water and a typical lipoid like lecithin. That is, for any series of lipoid-soluble compounds the narcotic action increases as the lipoid-solubility increases and the water-solubility decreases. These two solubilities usually show an in-

verse relation to each other. Now, the view that this form of anesthetic action depends essentially upon a modification of the cell-lipoids—which was put forward simultaneously and independently by Overton and Meyer—is undoubtedly well founded, and is accepted by most physiologists, even although in the absence of any definite and final knowledge of the physiological rôle of these cell-constituents it is far from being a complete theory of anesthesia. Such a theory would evidently involve a complete theory of stimulation, and this can hardly be said to exist as yet. It is, however, possible, I believe, to gain further insight into the nature of anesthetic action by combining the results of these and similar experimental studies of anesthesia with the results of certain more recent studies of the nature and conditions of the process of normal stimulation. It is necessary to form some clear conception of the nature of the changes involved in stimulation before we can profitably consider the question of just how the stimulation-process is modified by the presence of the anesthetic.

Before considering in more detail the mechanism of stimulation and of its modification by anesthetics, let us first consider briefly the nature of the physico-chemical constitution of the living cell, as more recent research has led us to conceive of it. This is a subject which is not easy to summarize, and on which much light remains to be thrown. It is clear, however, that the living protoplasm is not a homogeneous solution, but is a "polyphasic system"; i. e., a mixture consisting of various substances and solutions which are only partly miscible with one another, and are thus inter-related like the different phases of an emulsion or similar system. These several phases, which are partly solid, partly liquid, appear in each living cell to have a constant and definite arrangement, whose exact nature varies characteristically from

cell to cell. There appears typically to be a solid or semi-solid structural substratum consisting of colloidal material, most of which is in a water-swollen or hydrated state; in addition to this more fixed and permanent part of the cell-organization, numerous simpler substances are present—sugars, salts, amino-acids and others—largely in a state of simple aqueous solution, but probably partly adsorbed at the surfaces of the colloidal phases. There is evidence that it is by the oxidation of certain of these substances, especially sugar, rather than of the colloidal material, that most of the energy manifested in the cell-processes is set free. The colloidal substratum furnishes the conditions under which the energy-yielding oxidations and other metabolic changes take place, and apparently determines their course, character and velocity. The solid colloidal material of the cell may in one sense be considered as by-product of the metabolic activities of the protoplasm; it appears, once formed, to undergo itself relatively slight change, but to influence profoundly, by its presence and arrangement, the character of cell-metabolism.⁴ The colloids are of varied chemical nature; they are chiefly proteins and lipoids, and it is to be noted that they are built up by various forms of molecular union and polymerization from relatively simple substances furnished by the environment. This is true not only of plants, but also of the individual cells of higher animals, where the material which goes to form proteins reaches the cell in the form of amino-acids, or of simple polypeptides.⁵ In general it is from such amino-acids together with salts and carbohydrates that the cell builds up the colloids which form its char-

acteristic structural apparatus. This appears highly complex in some forms of irritable tissue, as in voluntary muscle; in others, as in nerve, the essential structure appears relatively simple. What we call the "structural organization" of the cell is merely another name for the physical characteristics and arrangement of the solid colloidal material.

However simply organized a cell may seem, there are certain elements of structure which appear always to be present, and to play a fundamentally important rôle in stimulation and in other life-processes. These are the *membranes*. Most, if not all, living cells are delimited from the medium in which they live by thin semi-permeable colloidal surface-films, the so-called plasma-membranes. Similar semi-permeable partitions are often found in the cell-interior, *e. g.*, about nuclei, vacuoles, chromatophores, and other structures. They appear to be formed of the same colloids as the other protoplasmic structures, namely, proteins and lipoids. These colloids, like many other organic substances, have, when dissolved in water, a marked influence in lowering the surface-tension of the solvent. Any substance thus acting tends, by the operation of Gibbs's principle, to collect or condense on the free surfaces; if the substance is colloidal in nature it may there pass out of solution and form a solid surface-film or membrane; and it is probably under conditions essentially like these that the cell-membranes are formed. Artificial membranes similar in many of their properties to the plasma or nuclear membranes of cells may be formed in protein solutions about droplets of chloroform, mercury or other water-immiscible substances. Now the plasma-membranes of irritable cells undoubtedly play a fundamentally important part in stimulation, as will be seen below, so that it will be necessary to consider first some of the essential properties of these

⁴ Cf. Child's interesting discussion of the relation between metabolism and structure in the *Journal of Morphology*, Vol. 22, 1911, p. 173.

⁵ Cf. Folin's recent papers in the *Journal of Biological Chemistry*.

membranes before passing to the consideration of the stimulation-process itself and its modification by anesthetics.

The plasma-membranes are typically *semi-permeable* structures—so much so that living cells form in many cases the most convenient and rapidly acting osmometers that we possess. If we place living cells, like plant-cells or blood corpuscles, in solutions of sugars, neutral salts and various other substances not in themselves immediately injurious to the cells, osmotic effects result from which the osmotic pressure of the solution relatively to that of the cell-contents can be estimated with great accuracy—as the researches of de Vries, Overton, Hedin and many others have shown. Two provisos are necessary in making use of living cells as osmometers: first, the dissolved substance must not by its own action impair the semi-permeability of the membrane, and second, it must not appreciably penetrate the membrane during the time occupied by the experiment. The plasma-membranes are in fact semi-permeable only in relation to certain classes of substances; towards others they show themselves freely permeable, and the character of these substances is important, because indication is thus afforded of the chemical nature of the materials composing the membranes. This is a matter of fundamental importance in the theory of anesthesia. Let us take for example a tissue composed of typical irritable cells, such as a frog's voluntary muscle. In studying the osmotic properties of this tissue, Overton found many years ago that the cells behaved in solutions of certain substances as if they were enclosed by strictly semi-permeable membranes; the chief of such substances are sugars, neutral salts, polyatomic alcohols like mannite, and amino-acids like glycocoll; but toward a large series of mainly organic substances, including alcohols, esters, aldehydes, hydrocarbon-deriva-

tives and others, the membranes behaved as if freely permeable. Thus in an $m/8$ (0.7 per cent.) solution of NaCl the muscle retains its weight unaltered, neither absorbing nor losing water; similarly in solutions of sugar, mannite or glycocoll of the same osmotic pressure (of about 6 atmospheres). But if to an $m/8$ NaCl solution we add (*e. g.*) alcohol in sufficient quantity to double the total osmotic pressure of the solution, it is found that there is no perceptible increase in its osmotic action on the muscle; in other words, the alcohol acts as if it entered the cell with the same readiness as the water. Many other substances show a similar power of freely entering the cell; others like urea, glycol and glycerine also enter, but more slowly. Similar observations in both animal and plant cells have shown that readiness of entrance into cells is a property that is closely correlated with solubility in fats or fat-like substances, including lipoids like lecithin. Substances not so soluble usually gain entrance slowly or imperceptibly. Overton drew from these facts the conclusion that the outer limiting layer or plasma-membrane of living cells consists in large part of lipoids, and that the characteristic osmotic properties of the cell depend on the presence of these substances in the membrane. Since substances that dissolve in lipoids will pass readily through lipid-impregnated partitions, this view explains why plasma-membranes are in fact readily permeable to such substances as a class. In its detailed application Overton's view has met with considerable opposition; thus, according to Overton, intra-vitam dyes like neutral red and methylene blue enter cells readily *because* of their solubility in lipoids; a certain number of exceptions to this rule have been pointed out by Ruhland and others; but in spite of these discrepancies there seems no doubt—when the whole of the evidence is considered—of the truth of Overton's

main contention that lipid-solubility strongly furthers the ready entrance of substances into cells. Traube believes that the degree of surface-activity, rather than of simple lipid-solubility, is the determining factor; this property shows a general parallelism with the lipid-water partition-coefficient, and hence also with the readiness of penetration. But there appear to be more exceptions to Traube's rule than to Overton's, while there are other and independent indications that the surface-films of cells are characteristically rich in lipoids; for instance, the fact that in the eggs of sea-urchins and other animals lipid-solvents are especially effective in causing the formation of fertilization membranes (a typical surface-effect) and in initiating cell-division; also the facts which I shall cite presently, showing that lipid-solvents are characteristically effective in altering the permeability of the plasma-membranes and in modifying their resistance to alteration by cytolytic substances.

I dwell upon these researches here because of the light which they throw on the question of the constitution of the plasma-membranes of cells, including those of the irritable tissues. They indicate that the membranes—and perhaps surface-structures in general—are especially rich in lipoids. Now lipid-soluble substances will tend, by the operation of the partition-law, to concentrate in the lipoids of the tissue; they will thus tend to gather in the membranes, and in so doing they will necessarily modify the physical state of these structures and so influence their physiological properties. We have already seen that the most striking effect which lipid-solvents produce on irritable tissues is to modify their irritability, and under certain conditions to suppress it altogether. This suggests that the membranes have a special relation to stimulation. We should expect on *a priori* grounds that the excitatory

apparatus of the cell should be externally situated; and we are thus led to inquire if there is other and independent evidence that the surface-films or plasma-membranes of the irritable elements play any such special part in stimulation.

There are in fact many indications that this is the case. Investigation of the conditions of electrical excitation—undertaken quite without reference to the problem we are considering—has shown that the semi-permeable membranes of irritable tissues are intimately concerned in stimulation. The first definite proof of this was brought forward in 1899 by Nernst. He was struck with the fact that Tesla currents (or alternating currents of high frequency) may be passed through irritable tissues (or through the human body) without causing stimulation; while if the frequency of the current is sufficiently lowered, but without altering its intensity, strong stimulation results. Now what does this mean? Evidently that the current must flow for a certain minimal time in a *constant direction* in order to stimulate. Mere conduction of a given quantity of electricity through an irritable tissue is not in itself sufficient to cause stimulation. There is some kind of cumulative effect depending on a steady flow in one direction. What physical peculiarities of the living tissue condition this remarkable peculiarity? Nernst pointed out that a living tissue in its relation to the electric current is an electrolytic conductor, which, however, is not homogeneous like an ordinary salt-solution, but peculiar in being sub-divided at intervals by semi-permeable partitions, the cell-membranes. When therefore the current starts to flow it carries as usual anions toward the anode and cations toward the cathode, but at the semi-permeable membranes this movement is blocked; the concentration of anions thus tends to rise above that of cations on the side of the membrane facing the cathode

and *vice versa*, and the above behavior of the tissue may be partly explained if we assume that these changes of concentration must reach a certain degree if stimulation is to result. For this effect time is required. Hence, if the current is reversed too soon, the stimulating effect is annulled, and the tissue remains unaffected. Now, if the assumption is true that stimulation is the expression of a change of electrical polarization, due to a change in the concentration of ions at the membranes, the time during which the current must flow in order to produce a given polarization-effect ought to correspond with that needed for a given stimulation-effect. Nernst's analysis shows that the polarizing action varies directly with the intensity of the current (*i. e.*, the quantity of electricity—*i. e.*, ions—transported in unit time) and with the square root of its duration ($S = Ki\sqrt{t}$), and observations on a variety of irritable tissues have shown that the stimulating action of a given current does vary in essentially this manner with the duration of its flow in one direction (*i. e.*, inversely with the square root of the number of alternations in the case of an alternating current). It seems clear then that electrical stimulation is dependent on the polarization-changes produced by the current at the semi-permeable membranes of the irritable elements.

One main result of these investigations is thus to localize the stimulating action of the current at the semi-permeable membranes, and to indicate that a change in the electrical polarization of these membranes is an essential feature of stimulation. It is evident that this result does not constitute a complete analysis of the nature of stimulation. But it indicates that some change in the membrane is essential to this process. An electrical variation accompanies every normal stimulation and undoubtedly forms an inseparable feature of the process, but

its conditions are still imperfectly understood. There is evidence, however, that it is associated with a definite alteration in the osmotic properties of the membrane. The electrical properties of irritable tissues like muscle indicate that during life there exists permanently in the resting cell a difference of potential, equal roughly to 1/10 volt, between the outer and inner surfaces of the plasma-membrane. When the uninjured outer surface of a muscle or nerve is connected through a galvanometer with the exposed interior of the elements (cut surface) a current, the so-called "demarcation-current," flows from exterior to interior, indicating that the outer surface of the cells or nerve fibers has a higher potential than the interior. This potential-difference appears dependent on the semi-permeability of the plasma-membrane; it is absent in dead cells whose plasma-membranes have lost their semi-permeability, and it is diminished by the application of poisons which impair the normal semi-permeability. Briefly, the demarcation-current potential appears in some way to be inseparably connected with this semi-permeability of the membrane. Ostwald in 1890 suggested a possible explanation of this condition when he pointed out that a membrane might become the seat of a potential difference by interfering unequally with the diffusion of the anions and cations of an electrolyte contained within the cell. If the plasma-membrane allowed cations to pass outward freely, but prevented the passage of anions, a state of things would be produced comparable to what we observe in living cells. But the actual conditions are probably more complex than this, and experimental substantiation of Ostwald's suggestion has not been satisfactory. The subject is evidently one requiring further investigation. In any case, however, the existence of the demarcation-current potential appears dependent on the semi-perme-

ability of the membrane, and whenever the latter undergoes marked increase of permeability, this potential is invariably decreased. The electrical variation or action-current which normally accompanies stimulation may be theoretically accounted for by assuming that at this time the membrane undergoes a decided but temporary increase in permeability, *i. e.*, loses the semi-permeable properties which it possesses during rest, and there is independent evidence that this change actually occurs during stimulation. If this is the case the plasma membrane is the seat of the most constant and characteristic manifestation of stimulation—the electrical variation or action-current. The membrane responds to the stimulating condition by suddenly changing its permeability and hence its electrical polarization.

We are thus brought to the conclusion that the plasma-membrane is characteristically and intimately concerned in the stimulation process. During stimulation it appears to undergo a sudden and quickly reversible increase of permeability. The electrical variation is one expression of this change, but there are others as well. Thus the movements of sensitive plants, which occur under the same conditions of stimulation as those of irritable animal tissues, are due to a collapse of turgid cells, consequent upon a sudden loss of the semi-permeable properties of the plasma-membranes enclosing the osmotically active solution or cell-sap. Here at least is one irritable tissue where the connection between permeability-increase and stimulation seems unmistakable. It might be held that the existence of special osmotic motor mechanisms in certain plants affords no indication of the nature of the conditions in irritable animal cells; but even in animals there are in some cases very clear indications that stimulation is constantly associated with an increase of permeability, as I shall shortly point out.

Evidence from various sides thus proves the participation of the plasma-membranes in the stimulation-process. Just why a change in the permeability and electrical polarization of the plasma-membrane should influence so profoundly the metabolic and other activities of the cell is naturally a far-reaching question requiring further investigation, but there are many reasons for believing that the primary or initiatory phase of the stimulation-process is a change of this nature.

Let us return now to the question of why anesthetics interfere with the stimulation-process. In the first place they can be shown experimentally to interfere with both of the above characteristic manifestations of stimulation, (1) the action-current and (2) the change of permeability. If these are the critical or primary events, on which the other effects following stimulation depend, it is evident that suppression of these must involve a suppression of the entire series of processes resulting from stimulation, including the oxidations, the contraction-changes and the other special features of the response.

That the action-current as well as the mechanical response of a muscle is suppressed by anesthetization has long been known. In nerve also anesthesia abolishes the action-current. Now, on the foregoing hypothesis, the electrical variation is the expression of some alteration in the plasma-membrane, involving a temporary increase of permeability. Höber has found that potassium salts, which deprive nerves of irritability and render them locally negative, cause at the same time a visible alteration in the axis-cylinders; these structures swell and stain more diffusely; he found further that these effects are checked or prevented if the nerves are first anesthetized with ethyl urethane. Experiments on voluntary muscle gave analogous results. If a frog's muscle is partly dipped into an

isotonic solution of a potassium or rubidium salt the tissue contracts somewhat and becomes locally negative; this effect is also inhibited or retarded in the presence of an anesthetic.* If the local negativity is the expression of a change produced by the salt in the colloids of the plasma-membrane, rendering the latter more permeable than before, Höber's results indicate that the anesthetic decreases the susceptibility to such changes of permeability. If this is the case we can partly understand why the anesthetized tissue becomes less susceptible to stimulation, since stimulation involves an increase of permeability.

Quite recently at Woods Hole I have investigated the question of the nature of anesthetic action in a somewhat different manner, using an organism which seems unusually well adapted to throw clear light on this subject. If an anesthetic acts by so modifying the plasma-membrane of the irritable cell as to render difficult or impossible the rapid variations of permeability which are essential to stimulation, it ought to act similarly on other cells, *i. e.*, it should protect these cells also against the action of permeability-increasing substances or agencies. If an organism can be found whose cells undergo immediate and obvious increase of permeability under conditions which at the same time cause stimulation, it should become possible to determine whether suppressing the stimulating action of a given agency is equivalent to a suppression of its permeability-increasing action. The two effects ought to show a definite parallelism if the above hypothesis is well-based. The organism which I have used is the larva of the marine annelid *Arenicola cristata*. This organism shows anatomical features of the required kind. It is a small, worm-like trochophore about 0.3 mm. long, swimming by two

ciliated rings and possessing a well-developed musculature of longitudinal fibers. The body cells are permeated with a yellow or brownish pigment, so that when the larvæ are collected in a dense mass (which can readily be done by taking advantage of their strong heliotropism) they appear dark brown in color. Now if such a mass of larvæ is brought suddenly into a pure isotonic NaCl solution, they instantly contract strongly and remain thus contracted for twenty or thirty seconds, after which they slowly relax. During the period of contraction the yellow pigment diffuses rapidly into the solution and colors the latter bright yellow; *i. e.*, strong stimulation of the muscle cells is associated with marked increase in the permeability of the pigment-containing cells. The cilia cease and undergo rapid disintegration at the same time. If now instead of using pure NaCl solution, we bring a similar mass of larvæ into NaCl solution to which a little calcium or magnesium chloride has been added, a strikingly different effect is seen. Stimulation is slight and transitory, there is no immediate loss of pigment, and ciliary action continues uninterrupted. The general toxic action of the NaCl is also greatly lessened. It can thus be shown that pure solutions of sodium salts cause strong stimulating and permeability-increasing effects, both of which are simultaneously prevented by the addition of a little calcium or other antitoxic salt. Prevention of permeability-increase runs parallel with prevention of stimulation. Magnesium salts in pure isotonic solution exhibit an action which is apparently the reverse of that shown by sodium salts. Larvæ brought into $m/3$ $MgCl_2$ show no stimulation, no loss of pigment or destruction of cilia, and little or no immediate injury. On the contrary all muscular movements cease in a few seconds and the larvæ remain permanently motionless during their stay in the solution (ex-

*Cf. Höber, *Pflüger's Archiv*, 1907, Vol. 120, p. 492.

cept for the cilia which remain active). The effect of the solution is reversible; on return after a few minutes to sea-water, the normal activities at once return. The magnesium salt shows typical anesthetic action, *i. e.*, it renders stimulation difficult or impossible. It also hinders increase of permeability. If larvæ that have lain in $m/3$ $MgCl_2$ for a few minutes are suddenly brought into $m/2$ $NaCl$, no immediate effect is seen—neither stimulation nor loss of pigment. The toxic action of the pure $NaCl$ is also much less than when the transfer to this solution is made directly from sea-water. In other words, the $MgCl_2$ renders the plasma-membranes resistant to the permeability-increasing or cytolytic action of the $NaCl$ solution; and at the same time it renders the irritable elements resistant to stimulation. The action of the $MgCl_2$ must depend on an alteration of the cell-surfaces, since this salt enters living cells with extreme slowness, if at all. We must conclude that in this instance at least the anesthetic action depends on a modification of the surface-layers or plasma-membranes of the irritable cells or elements.

I have found that lipoid-solvent anesthetics produce effects which are essentially identical with those of $MgCl_2$. The case of ethyl ether, the most widely used of all anesthetics, may serve as an illustration. In a .7 per cent. solution of ether in sea-water *Arenicola* larvæ immediately cease all muscular movements; the cilia show more resistance to anesthesia and remain active. If now the larvæ are transferred to $m/2$ $NaCl$ solution containing the same proportion of ether—so as to preserve the state of anesthesia—no contraction or loss of pigment follows, the cilia continue their activity, and the injurious action of the pure $NaCl$ is relatively slight. Bringing larvæ from normal sea water directly into ether-containing $NaCl$ solution also causes little or no stimulation or loss of pigment, and

the cilia and body cells are protected against the injurious action of the solution. Thus in the presence of the anesthetic the salt solution fails to show its normal stimulating and permeability-increasing action, and its toxic or cytolytic action is greatly diminished. Anti-stimulating and anti-cytolytic actions run parallel with each other.

I have studied the action of a large number of anesthetics in this manner. Those which promptly and completely anesthetize *Arenicola* larvæ in sea-water show, when dissolved in $NaCl$ solution in the proper proportions, effects which are essentially identical with those just described, though varying in degree with the different anesthetics. Alcohols (methyl, ethyl, propyl, butyl, amyl, capryl), the urethanes (methyl, ethyl, phenyl), other esters like ethyl nitrate, acetate, propionate, and compounds like chlorethone, acetanilide, paraldehyde, nitromethane, chloroform, acetonitrile, all decrease or prevent the stimulating and permeability-increasing action of pure $NaCl$ solutions when present in the concentrations which cause typical anesthesia in sea-water. They also show well-marked protective or anti-cytolytic action. Other anesthetics, among which are chloral hydrate, benzol, phenyl urea, and chloralose, act more slowly than those just mentioned, and if larvæ are brought suddenly into their solutions in $m/2$ $NaCl$, stimulation and loss of pigment occur very much as in the pure salt solution. A parallelism between permeability-increasing action and stimulating action is thus seen throughout. If the one effect is decreased or prevented so also is the other.

The exact concentrations most favorable for anesthesia and prevention of permeability-increase are characteristic for each substance and have to be determined empirically. In a series of homologous compounds, like the alcohols or the fatty acid esters, the

molecular anesthetic action increases rapidly with increase in molecular weight and in lipid-water partition-coefficient. The same is true for the vertebrate central nervous system, as Overton and Meyer have shown. Overton's observations on tadpoles show a close parallelism with my own in these respects. The concentrations required to anesthetize *Arenicola* larvæ are, however, higher in every case—usually three to five times higher—than for tadpoles. Possibly the higher salt content of the tissue-media in marine animals is responsible for these differences; the order of relative action is the same in both organisms.

To sum up—it would thus seem that anesthetics produce their essential effects by modifying the properties of the semi-permeable plasma-membranes of the irritable tissues, making these structures more resistant to changes of permeability than normally. Since variations of permeability are essential to stimulation, the irritable tissue is thus rendered temporarily insensitive or irresponsive.

How does the anesthetic produce these effects? Osterhout has recently shown that anesthetics decrease the electrical conductivity of plant tissues, apparently by decreasing the permeability of the plasma-membranes to ions;⁷ and it may be that in irritable animal tissues also the permeability normal to the membranes is similarly decreased during anesthesia. If the distinctive action of the anesthetics is to *decrease* permeability, its presence in the tissue will naturally oppose increase of permeability and hence interfere with stimulation. The osmotic motor mechanisms of plants, whose action depends on sudden increase of permeability, may in fact readily be rendered irresponsive by anesthetics, as Claude Bernard pointed out long ago in his classical lectures on the life-phenomena

common to animals and plants. Or the explanation may be somewhat different. The anesthetic may leave the resting permeability of the membrane the same as before—or perhaps may change it in either direction—but alter its properties so as to decrease the readiness with which the permeability is changed by other agencies acting on the membrane. Changes of condition, electrical or otherwise, that normally act as stimuli would then no longer affect the membrane, and would hence cease to stimulate. But whatever general interpretation we adopt, it is demonstrable that the properties of the membranes are altered during anesthesia in such a way as to make increase of permeability more difficult than in the normal sensitive state of the irritable tissue.

Is this the whole explanation of the anti-stimulating action of anesthetics? Nothing but further experimentation can answer such a question. Suppression of stimulation is however the essential effect to be explained. It must be remembered that any specific response to stimulation comprises a series of mutually interdependent processes, beginning with the one caused directly by the external agent, and ending with the special physiological activity, or response, characteristic of the tissue. It seems more likely that the anesthetic interferes with the *initial* process of such a series than with one occurring later—such as the increase in oxidation or other special effect. The evidence which I have cited indicates in fact that the primary process in stimulation is a *membrane-process*, and that it is this process which is modified by the anesthetic. This is why the succeeding and outwardly more evident effects of stimulation are also modified in the way that we observe.

It is well known that an influential group of physiologists, headed by Verworn, maintain that a suppression or prevention of

⁷ SCIENCE, Vol. 37, 1913, p. 111.

oxidation-processes is the essential basis of anesthesia. Various facts are adduced in support of this theory. During anesthesia the oxidative metabolism of the tissue is diminished. This fact in itself is equivocal; stimulation causes increased oxidations in many tissues, and suppression of stimulation prevents this effect along with the others. Lack of oxygen arrests many physiological activities that are dependent on its presence, but this fact again does not justify Verworn's identification of narcosis and asphyxia. Other facts seem more consistent with this view. Fröhlich and Heaton find that the recovery of nerves from anesthesia is imperfect or delayed in absence of oxygen; Ishikawa, another pupil of Verworn's, finds the same for *Amœbæ*; from which they conclude that suppression of oxidations is the essential feature of the condition. These observations merely show once more that the cell or tissue requires oxygen in order to exhibit its normal properties. Mansfeld finds that the concentration of anesthetic required to anesthetize tadpoles is less when oxygen is deficient than when it is abundant; *i. e.*, anesthesia and asphyxia show additive relations to each other. This again is equivocal. The action of nerve-cells is intimately dependent on a good supply of oxygen; when oxygen is deficient their excitability is lowered, and along with this the degree of anesthesia required to abolish excitability. Other parallels of similar nature seem open to objections of the same kind. On the other hand, nerve trunks resist the lack of oxygen or the presence of cyanide (which renders unavailable the oxygen present) remarkably well. Warburg finds that fertilized sea-urchin eggs anesthetized by phenyl urethane, so as to be incapable of cell-division, show nevertheless the same oxygen-consumption as the normal unanesthetized eggs. Again, lack of oxygen interferes only

gradually with the ciliary action in many organisms, while anesthetics in sufficient concentration arrest the movement instantly. It seems necessary to conclude from these facts that the essential action of the anesthetic is of a more general kind, and consists in incapacitating some mechanism which is essential to the normal activities of the cell, whether these immediately require oxygen or not.

The evidence which I have reviewed indicates either that this mechanism is the plasma-membrane itself, or that it is closely dependent on the condition of the plasma-membrane. Any condition that renders the membrane incapable of responding to changes of condition by rapid changes of permeability and of electrical polarization has an anesthetic influence. This modification in the properties of the membrane may be produced either by changing the general condition of the colloids forming it—as in the case of magnesium salts or electrolytes in general—or by specifically altering the state of the lipoid-components, as by organic anesthetics. It is impossible to say at present precisely why the solution of an anesthetic in the lipoids of the membrane should thus alter the properties of this structure. The nearest physico-chemical analogy seems to be the so-called "protective action" of colloids, as exemplified in those cases in which the presence of one colloid interferes with or prevents changes of aggregation-state in another, *e. g.*, when gelatine prevents the precipitation of colloidal gold or platinum by a neutral salt like sodium chloride. Apparently the lipoids are related to the other colloids of the membrane in such a manner that the condition of the lipoids affects the entire properties of the colloidal structure, and so determines the effect which an electrolyte like NaCl, or a stimulating condition like an electric shock or mechanical im-

paet, may have upon it. Hence when a lipoid-solvent acts upon the membrane, and dissolves in the lipoids of the latter, it may profoundly change the physical properties of the membrane and hence the responsiveness of the whole tissue or organism to stim-

ulation. On this view the membrane is a main controlling factor in cell-processes, and by changing its state we may alter the entire physiological activity of the cell.

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THE PHYSIOLOGY OF CELL-DIVISION

V. SUBSTITUTION OF ANESTHETICS FOR HYPERTONIC SEA-WATER AND CYANIDE IN ARTIFICIAL PARTHENOGENESIS IN STARFISH EGGS

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I. INTRODUCTION

The problem of fertilization presents various analogies with the problem of stimulation—some superficial, others indicating that the two processes possess certain fundamental physiological features in common. In both cases the cell gives a qualitatively constant or specific response to a change of condition which itself need not be specific. Thus, a muscle contracts when artificially excited by chemical, electrical, or mechanical stimuli which never act upon it in the intact organism, in the same manner as in response to the normal physiological stimulus or nerve-impulse. Similarly, the egg-cell begins its characteristic cycle of cell divisions after subjection to various artificial forms of treatment, as well as after the normal contact and entrance of the spermatozoon. It is a special peculiarity of the egg-cell that these cell-divisions, once started, continue automatically in a regular and predetermined rhythm and are associated with the definitely directed and progressive processes of growth and differentiation which constitute development. But this process, however complex, is none the less the constant and distinctive mode of response of the egg-cell, just as contraction is of the muscle-cell. A relatively slight and non-specific disturbance initiates in either type of cell its own characteristic and complex type of physiological activity.

There are indications that something more is involved here than a mere formal resemblance or analogy. In stimulation a temporary change in the electrical polarization of the limiting membrane of the irritable element is the essential or critical event.¹ This change—so far as the present evidence extends—appears to be conditioned by a temporary variation in the properties of the limiting membrane. Apparently the latter undergoes a rapid and automatically reversible increase of permeability; hence the electrical polarization of the membrane, which in the resting cell is a function of the normal semi-permeability,² is temporarily diminished; the characteristic electrical variation of stimulation is an expression of this change. In other words, the membrane temporarily loses its semi-permeability during stimulation. The evidence of this is largely indirect, in the nature of the case, but fortunately one irritable tissue is known in which there is clear and unequivocal indication that the membranes lose their semi-permeability during excitation; this is the osmotic motor mechanism of sensitive plants, where movement results from a sudden loss of turgor. Since turgor is dependent on semi-permeability, its sudden loss can only mean loss of semi-permeability. Evidence that similar conditions exist in irritable animal tissues is seen in the increase of electrical conductivity of muscle during excitation,³ and in the temporary loss of irritability (the so-called refractory period) which accompanies the rising phase of the electrical variation: it is clear that if the membranes of the irritable tissue lose their semi-permeability during stimulation, any repetition of stimulation will be impossible until the semi-permeability necessary to this process is regained, since relative impermeability to ions is a necessary condition of the polarizing action of the electrical current, and it is this action—as Nernst has shown—which is the essential in electrical stimulation. I have also adduced evidence in favor

¹ This is the necessary inference from the studies of Nernst and his successors on electrical stimulation.

² Increase of permeability, however induced, decreases or abolishes the demarcation-current potential.

³ Cf. McClendon, *American Journal of Physiology*, 1912, vol. 29, p. 302; Galeotti, *Zentralblatt für Physiologie*, 1912, vol. 26, p. 536.

of this general theory from observations on the pigmented larvae of *Arenicola*, where stimulating solutions, for example, 0.55m NaCl, cause visible increase of permeability; stimulation and permeability-increase show an unmistakable parallelism in this organism, and both processes are simultaneously decreased or prevented by the same agencies, such as calcium or magnesium salts and anesthetics.⁴

In fertilization also the primary change is a surface-change, which almost certainly involves an increase in the permeability of the plasma membrane. Loeb's researches have demonstrated the general effectiveness of cytolytic substance in initiating cleavage in sea-urchin eggs.⁵ Pure solutions of neutral sodium and potassium salts have the same effect, which is the more marked the more energetic the permeability-increasing action, and is inhibited by salts like calcium or magnesium chloride which check or prevent this action.⁶ The egg-cell immediately after normal fertilization shows increased electrical conductivity⁷ and increased permeability to substances like sugar and alkali,⁸ as well as to its own pigment.⁹ All of these facts indicate that the fertilization-process involves an initial increase in permeability; such a change ought theoretically to be accompanied by a change in the electrical polarization of the membrane, similar to that which, on the membrane theory, conditions the action-current in irritable tissues; and Miss Hyde's observations on *Fundulus* eggs¹⁰ indicate that this is in fact the case. A depolarization-process thus probably accompanies the initial stage of fertilization, as well as of stimulation, and it is highly probably that it forms the critical or initiatory event in this process also.

⁴ I have discussed this subject in fuller detail in a recent paper in the *American Journal of Physiology*, 1911, vol. 28, p. 197.

⁵ J. Loeb, *Chemische Entwicklungserregung des tierischen Eies*, and his numerous earlier papers there cited.

⁶ R. S. Lillie, *American Journal of Physiology*, 1911, vol. 27, p. 289. *Journal of Morphology*, 1911, vol. 22, p. 695.

⁷ McClendon, *American Journal of Physiology*, 1910, vol. 27, p. 240

⁸ E. N. Harvey, *Science*, N. S. 1910, vol. 32, p. 565. *Journal of Experimental Zoology*, 1911, vol. 10, p. 547.

⁹ Lyon and Shackell, *Science*, N. S., 1910, vol. 32, p. 249.

¹⁰ I. H. Hyde *American Journal of Physiology*, 1904, vol. 12, p. 241.

If this is true, an externally induced depolarization ought to initiate cell-division in unfertilized eggs; the facts of electrical parthenogenesis thus favor this view. It is well known that cytolytic substances cause in muscle an electrical variation or 'injury-current,' similar in direction to the action-current, and indicating—according to the membrane theory—increased permeability of the membranes; the same change presumably occurs in egg-cells treated with cytolytic substances. There seems thus to be good reason for assuming that the initial event in fertilization, as in stimulation, is a depolarization-process, which is conditioned by a temporary increase in the permeability of the plasma membrane. Since this initial depolarization is in both cases reversible, we may conclude that the primary phases of both processes—those in which the membranes are concerned—are essentially similar and differ chiefly in their time-relations, the latent period being relatively long and the rate of the electrical variation relatively slow in egg-cells.¹¹ There is also reason to believe not only that a depolarization-change is associated with the fertilization-process, but that a rhythm of alternating polarization and depolarization is similarly associated with the rhythm of cleavage.¹² The appearance and reappearance of the characteristic system of cytoplasmic relations, coincidently with the rhythm of cleavage, seems satisfactorily accounted for on the assumption of an alternating polarization and depolarization of the plasma membrane; and the existence in sea-urchin eggs of a parallel rhythm of carbon dioxide production and of susceptibility to poisons¹³ constitutes a further and independent indication of a rhythmical variation of permeability.

In stimulation the initial depolarization, corresponding to the rising phase of the action-current curve, is promptly and completely reversible, and the same—with characteristic differences in time-relations—is probably true of the fertilized egg-cell.

¹¹ The rate of electrical variation varies widely in different tissues, being most rapid for nerve and least rapid for slowly responding tissues like smooth muscle and heart muscle.

¹² R. S. Lillie, *Biological Bulletin*, 1909, vol. 17, p. 207. *American Journal of Physiology*, 1910, vol. 26, p. 126.

¹³ Lyon, *American Journal of Physiology*, 1902, vol. 7, p. 56 and 1904, vol. 11, p. 52.

A corresponding reversibility in the associated permeability-change is thus implied. Now, the initial cytolytic process caused in sea-urchin eggs by fatty acids or other cytolytic substances appears to be imperfectly reversible unless the egg is afterwards subjected to a second treatment, which consists in exposure for a certain time to oxygen-containing hypertonic sea-water (for twenty to thirty minutes at 20°), or to cold (for several hours) or to cyanide-containing sea-water (for several hours) Sea-urchin eggs subjected to a simple membrane-forming treatment and then returned to sea-water typically undergo cytolysis, usually after some irregular form-change with perhaps an occasional cleavage. But after appropriate treatment with hypertonic sea-water, cold, or cyanide, many of these eggs are restored to an essentially normal state and proceed normally with their development.¹⁴ The necessity of such after-treatment, following the initial membranolytic or permeability-increasing process appears, however, to vary widely in different eggs. Sea-urchin eggs are characterized by a certain inertia or inability to recover a normal condition without such supplementary treatment. Starfish eggs, on the contrary, may develop normally in a large proportion of cases after simple membrane-formation by heat or fatty acid.¹⁵ In other eggs a local mechanical irritation may also be followed by normal development, as in Bataillon's experiments with amphibian eggs.¹⁶ In some of the experiments described in the present paper the starfish eggs were found to develop more favorably *without* the after-treatment with hypertonic sea-water, cyanide, or anesthetics, than with it. In these cases it is to be assumed that the egg spontaneously reverts to the normal condition of semi-permeability, and hence is enabled to proceed normally with its development. Such spontaneous recovery of the normal properties after artificial membrane-formation rarely occurs with sea-urchin eggs, where the after-treatment

¹⁴ Cf. J. Loeb, loc. cit.

¹⁵ See below. Cf. also my earlier paper on parthenogenesis by temporary warming, *Journal of Experimental Zoölogy*, 1908, vol. 5, p. 375.

¹⁶ Bataillon, *Archives de Zoölogie expérimentale et générale*, 1910, Ser. 5, vol. 6, p. 101.

is indispensable to the production of a large proportion of larvae; if this treatment is omitted the vast majority of eggs invariably undergo cytolysis.

These conditions suggest that in the sea-urchin egg the cytolysis following simple membrane-formation is an expression of the undue persistence of a condition of increased permeability; and that the after-treatment serves essentially to bring the permeability again to the norm.¹⁷ In the normal egg a curve of polarization-change (dependent on permeability-change) of the following general form probably follows the entrance of the spermatozoon; there is an initial depolarization resulting from the increase of permeability, and lasting for perhaps fifteen minutes; following this the permeability and the correlative polarization return to or toward the original condition, where they remain unchanged until the period of the first cleavage, when a second temporary depolarization takes place; a similar change recurs with each succeeding cleavage. In parthenogenetic fertilization with a cytolytic agent it is to be assumed that the initial treatment also causes an increase of permeability with accompanying depolarization, from which however the egg recovers only imperfectly unless subjected to a second treatment whose general effect on permeability is of the reverse kind, that is, of a kind tending to restore the original semi-permeability of the membrane. On this hypothesis the beneficial effect of the after-treatment depends on what may be broadly characterized as an *anti-cytolytic* action. The total process thus consists in a preliminary permeability-increasing treatment—equivalent to cytolytic if the condition is not soon reversed—followed after the proper interval by one whose general effect is permeability-decreasing or anti-cytolytic. If this is true it ought to be possible to substitute for the after exposure to hypertonic sea water or other favorable condition a treatment with other substances or agencies whose general effect is to *decrease* the permeability of the plasma membrane or to oppose any further increase of permeability. Such treatment ought to check or prevent the progressive breakdown or cytolysis otherwise following the

¹⁷ Cf. R. S. Lillie, *American Journal of Physiology*, 1911, vol. 28, p. 285; Godlewski, *Archiv für Entwicklungsmechanik*, 1911, vol. 33, p. 225.

simple membrane-formation, and thus enable a larger proportion of eggs to recover the normal state and proceed with their development.

During the summer of 1912 at Woods Hole I have experimented from this point of view with solutions of various anesthetics. Substances of this class have been found to check the permeability-increasing action of pure isotonic solutions of sodium and potassium salts on sea-urchin and starfish eggs. These anti-cytolytic effects are seen in certain definite concentrations, which correspond closely with those which anesthetize the neuromuscular system of marine organisms like *Arenicola* larvae; in these concentrations anesthetics have the general effect of rendering the plasma membranes of cells—egg-cells, muscle cells, pigment cells, as well as structures like cilia—more resistant than normally to agencies which tend to increase permeability (or cause breakdown in the case of cilia).¹⁸ Anesthetics thus counteract the action of permeability-increasing agencies, and it is probably for this reason that they prevent stimulation (which involves temporary increase of permeability) as well as retard cytolysis. In some cases they have been definitely shown to decrease the normal permeability of the plasma-membranes.¹⁹ The alterations which they induce in the properties of the membranes are thus, generally speaking, opposite in kind to those caused by permeability-increasing or cytolytic agencies. We should thus expect that if, as above assumed, the plasma membrane of the egg is deprived of its normal semi-permeability by the membrane-forming process—and is thus brought into a state which eventually leads to cytolysis—an after-treatment with anesthetics will tend to restore the original semi-permeability and will thus render the condition of the egg more favorable to the continuance of development. Solutions of anesthetics, in other words, ought to produce effects similar to those of hypertonic sea-water or cyanide.

¹⁸ Cf. my recent papers in the *American Journal of Physiology* on antagonisms between salts and anesthetics: 1912, vol. 29, p. 372; vol. 30, p. 1; 1913, vol. 31, p. 255.

¹⁹ Cf. the recent experiments of Osterhout, *Science*, 1913, N. S., vol. 37, p. 111; also my observations on *Arenicola* larvae; *American Journal of Physiology*, 1909, vol. 24, pp. 25 seq.

In the experiments about to be described I have found that anesthetics produce very definite effects of this kind with starfish eggs. Unfertilized mature eggs of *Asterias forbesii* treated briefly with isotonic NaCl solution, dilute fatty acid solution, or warm sea-water (35°), returned to sea-water for ten minutes, and then exposed for half an hour to sea-water containing a favorable anesthetic in appropriate concentration, yield a large proportion—in some cases 80 to 90 per cent—of active larvae. Such after-treatment is often more favorable than that with hypertonic sea-water. With *Arbacia* eggs, on the other hand, the results of similar experiments have been essentially negative. So far I have been unable to produce in these eggs any marked increase in the proportion developing to a larval stage by any method other than the use of hypertonic sea-water. Cyanide, in my experience, has proved only slightly effective. After-treatment with sea-water containing an increased proportion of magnesium and calcium salts (without altering the osmotic pressure), or with sea-water containing anesthetics (as in the solutions used below), is ineffective with *Arbacia* eggs. Just why the two species should thus differ cannot definitely be said at present; in general the starfish egg is the more responsive of the two, and may be made to form membranes and develop by various methods which have no effect on the *Arbacia* egg;²⁰ it is also more variable in its behavior and less resistant to injurious influences. It is significant that the plasma membranes of starfish eggs are much less resistant to the permeability-increasing action of salt-solutions than are those of *Arbacia* eggs,²¹ and the greater effectiveness of after-treatment with anesthetics may possibly indicate that the membranes undergo the reverse kind of modification (permeability-decrease) also with greater readiness. The difference in the degree of responsiveness would on this view be an expression of differences in the properties of the plasma membranes; these differences indicate differences of protoplasmic composition, since the membrane is presumably a haptogen film

²⁰ Brief warming, shaking, isotonic NaCl solutions, weak solutions of mineral acids, exposure to cold.

²¹ Cf. my papers in the *American Journal of Physiology*, 1910, vol. 26, p. 125; 1911, vol. 27, p. 289.

formed by a surface-condensation of certain protoplasmic constituents, particularly those which lower surface tension. The starfish egg is deficient in cholesterin according to Mathews,²² and this circumstance may be important, since the presence of cholesterin seems in general to render plasma membranes relatively resistant to chemical alteration.²³ The plasma membrane of the mature starfish egg seems unusually sensitive to changes in the surrounding medium, and the responsiveness of these eggs to the above form of after-treatment is probably an expression of this peculiarity. The experiments now about to be described relate entirely to starfish eggs.

II. EXPERIMENTAL

In all the experiments described below the unfertilized eggs of starfish (*Asterias forbesii* and *vulgaris*) were used.²⁴ The eggs were first subjected to a membrane-forming treatment; then, after an interval of ten minutes in normal sea-water, they were exposed for thirty minutes to sea-water containing anesthetics in the concentrations given below. For comparison, part of the eggs remained in sea-water without after-treatment, and part were exposed for thirty minutes to hypertonic sea-water and to sea-water containing potassium cyanide in m/1000 concentration. The eggs were then returned to normal sea-water and on the following day the proportion developing to a free-swimming larval stage was approximately determined.

²² A. P. Mathews, unpublished observations made at Woods Hole.

²³ Cf. Höber, *Physikalische Chemie der Zelle und der Gewebe*. 3rd Ed., 1911, p. 232.

²⁴ In 1912 good starfish eggs were abundant at Woods Hole throughout the summer. A difference was observable between the eggs obtained toward the middle and end of June, and those obtained later (from July on). The latter showed the more regular behavior and were in general more responsive, while eggs obtained towards the end of June were frequently resistant to fertilization and showed irregularities of behavior similar to those described in my recent paper in the *Biological Bulletin* (1912, vol. 22, p. 328). Two maxima of egg-production are thus indicated, which probably correspond to the two specific types, *A. forbesii* and *A. vulgaris*, recognized as occurring in this region. In the experiments described below the majority of the eggs showed normal behavior in regard to post-maturational cytolysis and response to fertilization.

Three types of artificial membrane-forming treatment were employed: (1) exposure for five minutes to pure isotonic sodium chloride solution (0.55m. NaCl); (2) exposure for one to two minutes to a weak solution of a fatty acid (acetic or butyric) in sea-water; and (3) exposure to warm sea-water (at 35°) for seventy seconds.

In the majority of experiments the eggs were thus treated at about the time of separation of the first polar body—that is, from one to one-and-a-quarter hours after removal from the animals—this being the time at which the eggs respond most favorably to either normal or parthenogenetic fertilization. In a number of instances the same treatment was applied also after maturation was complete, at three to four hours after removal. Of eggs treated at this time the proportion reaching larval stages is typically much smaller than in the first class of cases; this decrease in responsiveness to artificial treatment is probably correlated with the decreased susceptibility to sperm-fertilization which appears at this time;²⁵ the unfertilized eggs seem then normally to enter on a refractory or relatively unresponsive phase in the life-cycle. In several series of experiments, however, development proved more favorable in eggs which were treated after maturation was complete; this occurred chiefly in abnormal lots of eggs which exhibited delay in the post-maturational cytolysis and imperfect development after sperm-fertilization. In all those experiments where eggs treated two or three hours after maturation formed a considerable proportion of larvae (10–20 per cent), sodium chloride solution was the membrane-forming agent; this solution appears to act more energetically than the other two agents and is possibly for this reason better able to overcome the increased resistance which the eggs show at this stage. .

The experiments with each of the above types of membrane-forming treatment will now be described separately in some detail.

²⁵ R. S. Lillie, *Journal of Experimental Zoölogy*, 1908, vol. 5, p. 411.

Series I. Treatment with anesthetics after membrane-formation by isotonic sodium chloride solution

Brief exposure (five to ten minutes) to 0.55 m. NaCl is typically followed by membrane-formation in a considerable proportion of eggs, of which a minority develop to a larval stage.²⁶ This mode of treatment is less favorable than exposure to acid-containing sea-water or temporary warming, and even when a favorable after-treatment is used the proportion of eggs reaching larval stages rarely exceeds 20 or 30 per cent. Presumably the initial cytolytic action of this solution is too energetic to be readily counteracted or reversed by the subsequent anti-cytolytic treatment.

If eggs thus treated with salt solution are afterwards exposed for half an hour to hypertonic sea-water or cyanide solution, or sea-water containing anesthetics in appropriate concentrations, the proportion of eggs forming blastulae is typically and often markedly increased. The results of a series of such experiments are summarized in table 1.

In this series each form of after-treatment caused well-marked increase in the proportion of eggs developing to a blastula stage. It is to be noted that hypertonic sea-water was less effective than the other solutions; this is very generally the case with starfish eggs. After-treatment with hypertonic sea-water following membrane-formation by salt-solution appears often to be more effective with eggs treated *after* the completion of maturation than before. In three series where eggs from the same lots were thus treated both (a) during maturation and (b) three hours afterward, the proportions forming larvae were: (a) < 1 per cent, (b) 20–25 per cent; (a) *ca.* 1 per cent; (b) *ca.* 20 per cent; (a) 1 per cent; (b) *ca.* 10 per cent. No such differences were ever observed with hypertonic sea-water used after the other two membrane-forming agents. Cyanide and the anesthetics show well-marked action in the above experiments. In the series shown in table 2 the effects of after-treatment with alcohols (ethyl, propyl, butyl, and amyl) are illustrated.

²⁶ American Journal of Physiology, 1910, vol. 26, p. 119.

TABLE 1

July 4, 1912: 10.30 a.m. The eggs from several starfish were placed, about one-and-a-quarter hours after removal from the animals, in 0.55 m. NaCl solution. After five minutes in this solution they were returned to seawater. Part of the eggs remained permanently in sea-water (for control). The rest, ten minutes later were placed for thirty minutes in the several solutions of the series; they were then returned to sea-water. Next morning the proportion of mature eggs that had formed blastulae was approximately estimated. The results were as follows:

AFTER-TREATMENT	CONDITION NEXT MORNING
1. None (Control treated with 0.55m. NaCl alone)	Only one living blastula found; almost all eggs dead
2. Hypertonic sea-water (250 cc. sea-water + 30 cc. 2.5m NaCl)	Blastulae few though increased in number,—ca. 1 % of mature eggs
3. Sea-water + m/1000 KCN	Marked increase in blastulae: 25-30 % of mature eggs
4. Sea-water + 0.6 vol. % ether	Well-marked increase: ca. 10-15 %
5. Sea-water + 0.1 % chloral hydrate	Well-marked increase: ca. 10-15 %
6. Sea-water + 0.2 % ethyl urethane	Less favorable: ca. 2-4 %
7. Sea-water one-tenth saturated with chloretone	Considerable increase: ca. 5 %

Fertilized control. Eggs fertilized one-and-a-quarter hours after removal yielded a large proportion of normal larvae.

Unfertilized control. Eggs left in sea-water without treatment: next morning all mature eggs are dead and coagulated, without membranes or cleavage.

TABLE 2

July 6: 3.38 p.m. Eggs were placed about one hour after removal from the animals in 0.55m NaCl solution for five minutes, then returned to sea-water and after ten minutes placed in the following solutions for thirty minutes, then again returned to sea-water. The results were as follows:

AFTER-TREATMENT	PROPORTION OF MATURE EGGS FORMING LARVAE
1. None (control treated with 0.55 m NaCl alone)	Proportion small (ca 1 %)
2. Hypertonic sea-water	Well-marked increase: 10-15 %
3. Sea-water + m/1000 KCN	Well-marked increase: 10-15 %
4. Sea-water + 5 v. % ethyl alcohol	Like 2 and 3: 10-15 %
5. Sea-water + 2.5 v. % n-propyl alcohol	Little if any increase <1 %
6. Sea-water + 1 v. % n-butyl alcohol	Fair increase: ca. 5 %
7. Sea-water + 0.25 v. % n-amyl alcohol	Well-marked increase: 10-15 %

Controls. Eggs fertilized one hour after removal developed normally. Unfertilized eggs were all dead and coagulated next morning.

In this series the alcohols, with the exception of propyl alcohol, showed well-marked though not pronounced action. The concentrations in which the alcohols act most favorably correspond closely with the minimal concentrations for producing muscular anesthesia in *Arenicola* larvae.²⁷ The above solutions of propyl and butyl alcohols appear to be somewhat above the optimal strength; in most of the following experiments weaker solutions, 2 v. per cent and 0.8 v. per cent respectively, were used. The following series (table 3) shows results similar to the above.

TABLE 3

July 27: The treatment was the same as in the preceding series

AFTER-TREATMENT	PROPORTION OF EGGS FORMING LARVAE
1. None (treated with 0.55m NaCl alone)	Very few form larvae: < 1 %
2. Hypertonic sea-water	Well-marked increase: 10-15 %
3. Sea-water + m/1000 KCN	Marked increase: 40-50 %
4. Sea-water + 5 v. % ethyl alcohol	Marked increase: 20-25 %
5. Sea-water + 2 v. % n-propyl alcohol	Marked increase: 30-40 %
6. Sea-water + 0.8 v. % n-butyl alcohol	Less favorable: 15-20 %
7. Sea-water + 0.25 v. % n-amyl alcohol	Still less favorable: 5-10 %

Controls. Nearly all eggs fertilized one hour after removal develop normally. Unfertilized eggs undergo normal coagulation without membrane-formation or cleavage.

In the experiments with sodium chloride solution, followed by after-treatment as above, the proportion of eggs developing to a larval stage was in some cases considerable—at times reaching 30 or 40 per cent—but never very high. In several other similar experiments less favorable results were obtained; usually the after-treatment caused a definite increase in larvae, but exceptions to this rule sometimes occurred, especially when the eggs were in any way abnormal. As already mentioned, sodium chloride solution gives in general less favorable results than the other two membrane-forming agents, probably because the action of the salt is too energetic and tends to produce effects not readily reversible by the subsequent action of the anesthetic, hypertonic sea-water, or cyanide. The experiments with fatty acid about

²⁷ American Journal of Physiology, 1913, vol. 31, p. 264.

to be described are more favorable and in some cases exhibit a close approach to the results of normal fertilization.

Series II. Treatment with anesthetics after membrane-formation by fatty acids

In this series the results of after-treatment with anesthetics were more decidedly and uniformly favorable than in the series with sodium chloride solution. The proportion of eggs forming larvae after such treatment is almost invariably much higher than after the simple membrane-formation with acid, although to this rule also there are certain exceptions, the more significant of which are considered below. Experiments with acetic acid solution followed by anesthetics were performed last summer with eighteen different lots of eggs at different times; in four of these series butyric acid was also used, making a total of twenty-two series of experiments with fatty acid. As a rule, starfish eggs treated with acid alone, without after-treatment of any kind, yield only a small proportion of larvae, rarely exceeding 10 per cent; this was the case in twenty out of the twenty-two series; in all of those series where the behavior of the control eggs was normal (seventeen in all) the after-treatment led to a marked increase in the proportion of favorably developing eggs, in some cases eighty per cent or more of the eggs forming active blastulae. In three series out of the twenty the eggs were unfavorable and yielded only a few larvae after normal fertilization, and correspondingly the proportion of parthenogenetically developing eggs was small; in two out of these three series the after-treatment with alcohol caused a definite though slight increase in the proportion of larvae; in the third the increase was doubtful. The remaining two series showed unusual behavior of a quite different kind; the eggs treated with fatty acid without any after-treatment yielded a high proportion of larvae, and the after-treatment appeared rather injurious than beneficial; these series will be discussed more fully below.

Last summer six series of experiments were performed with the solutions of table 4. Five of these series were carried out within a single week toward the end of June, and the sixth a few

days later. The table gives a summarized account of the results of all six series.

The results of these experiments show considerable uniformity. The number of larvae resulting from acid treatment alone was small, and each form of after-treatment caused a decided increase in the proportion of eggs forming larvae. It is noteworthy that hypertonic sea-water is on the whole less favorable than the other solutions. Cyanide and chloral hydrate show somewhat better results than the other solutions; this, however, is not always the case, as the following experiments with alcohols will illustrate.

TABLE 4

In each series the eggs were exposed, about one hour after removal from the animals, to a solution containing 6 cc. n/10 acetic acid, plus 100 cc. sea-water, for one to two minutes. Ten minutes later they were brought into the several solutions of the series, where they remained for thirty minutes; they were then returned to sea-water. Part of the acid-treated eggs received no after-treatment (for control). The hypertonic sea-water consisted in all cases of a mixture of 250 cc. sea-water plus 30 cc. 2.5m NaCl. The solutions (in sea-water) of cyanide and anaesthetics were kept in corked flasks. The results are given in approximate percentages of mature eggs forming swimming larvae (blastulae or gastrulae), under the date of the experiment. The condition of the control eggs, sperm-fertilized and unfertilized, is recorded in each case.

AFTER-TREATMENT	RESULTS					
	June 25	June 26	June 27	June 28	June 30	July 4
1. None (acid s.w. alone).....	ca. 1-2%	1-2%	1% or less	<1%	<1%	ca. 1%
2. Hypertonic sea-water.....	ca. 5%	ca. 5%	10-15%	ca. 10%	ca. 5%	5-10%
3. m/1000 KCN.....	ca. 20%	10-15%	20-25%	20-30%	<5%	30-40%
4. 0.6 v. % ether.....	10-15%	10-15%	<5%	ca. 10%	ca. 10%	25-35%
5. 0.1 % chloral.....	25-35%	>50%	10-15%	ca. 10%	<5%	25-35%
6. 0.2 % urethane....	10-15%	5-10%	ca. 10%	ca. 10%	< 5%	ca. 25%
7. 1/10 saturated chloretone.....	15-20%	15-20%	ca. 10%	ca. 10%	<5%	ca. 25-30%
Condition of control eggs.....	Normal	Partly resistant; good proportion normal	Normal	Partly resistant; majority normal	Considerable proportion resistant	Majority normal

In the series of June 30, and to a lesser degree of June 28, the increase produced by anesthetics was relatively slight. These eggs showed certain other abnormalities of behavior; they were partly resistant to fertilization, and in many the post-maturational cytolysis was delayed.²⁸ They also showed exceptional behavior in another respect; eggs treated in the above manner two hours after maturation was complete gave a larger proportion of larvae than those treated during the maturation period. Some instances of similarly abnormal behavior were cited above in the case of eggs treated with sodium chloride solution; in these cases also the eggs responded less favorably to a parthenogenetic treatment applied during the maturation-period than to one applied later. An abnormally lowered responsiveness during the maturation period seems thus often to be associated with an abnormally increased responsiveness later. The presence of a considerable proportion of abnormal eggs in the experiments of June 28 and 30 probably accounts for the relatively unfavorable character of the result on those days.

Effects of treatment with solutions of cyanide and anesthetics without previous membrane-formation. Experiments were performed to determine what effect the above forms of after-treatment, *acting alone*, have upon unfertilized starfish eggs. The results of these experiments show clearly that the treatment with these solutions must be *superposed* on the preliminary membrane-forming treatment in order to produce their characteristic effects. When acting alone they are almost or quite ineffective in inducing development, or what effect they have is confined to a small proportion of eggs, presumably the abnormal minority almost invariably present in lots of starfish eggs. The following experiments will illustrate: in the series of June 25 part of the eggs were exposed to hypertonic sea-water, cyanide and anesthetics at the same time as the others, but without having been treated previously with fatty acid. The results are summarized in table 5.

Several other similar series of experiments (June 20, 21, 23, 24) gave essentially the same results. Exposure to hypertonic

²⁸ For an account of this condition, cf. Biological Bulletin, 1912, vol. 22, p. 328.

sea-water for thirty minutes usually caused considerable membrane-formation and cleavage, and in a few cases gave a small proportion of blastulae. Ether showed a similar though less marked action; the proportion of eggs affected was in all cases small. With cyanide, chloral hydrate, urethane, and chloretone there was never any indication of membrane-formation or cleavage in eggs treated during the maturation period. Some slight effect however was seen in eggs exposed in the later period, and in three series a few eggs treated with chloretone, urethane, and chloral hydrate formed larvae, though the great majority remained unchanged. There was no action with cyanide. Apparently the conditions of response to the membrane-forming treatment undergo a certain change after maturation is complete: the increased resistance to normal fertilization then appearing is associated with a change in the effects produced by the above solutions of anesthetics. The existence of a certain proportion of eggs showing a behavior more or less divergent from the norm

TABLE 5

June 25, 1912: Unfertilized starfish eggs of the same lot were placed in the following solutions at two different intervals after removal from the animals; part A about one-and-a-quarter hours after removal, part B about two hours later. After remaining in the solutions for thirty minutes they were returned to sea-water. The condition of the eggs next day was as follows:

SOLUTION	A (EXPOSED 1½ H. AFTER REMOVAL)	B (EXPOSED 3½ H. AFTER REMOVAL)
1. Hypertonic sea-water.	About half the eggs show membrane-formation; the rest have coagulated unchanged. ¹ No blastulae	Majority coagulated unchanged; no blastulae
2. m/1000 KCN.....	No effect. All coagulated unchanged	All coagulated unchanged
3. 0.6 v. % ether.....	Almost all coagulated unchanged. A few membranes	Great majority coagulated unchanged; a few blastulae present
4. 0.1 % chloral hydrate.	All coagulated unchanged	All coagulated unchanged
5. 0.2 % urethane.....	All coagulated unchanged	All coagulated unchanged
6. 1/10 saturated chloretone.....	All coagulated unchanged	All coagulated unchanged

¹ That is, show the same condition as the untreated control eggs—dead and coagulated without membrane-formation or cleavage.

must also always be allowed for in lots of starfish eggs, which show marked contrast to the regularity and uniformity of behavior characteristic of *Arbacia* eggs.

Since treatment with anesthetics and cyanide alone, without preliminary membrane-formation, has typically no effect in initiating the development of starfish eggs, while after the membranes have been formed the same treatment greatly increases the proportion of eggs that develop favorably, it is to be inferred that the essential physiological effect of this treatment is of a kind quite different from that of the initial membrane-forming treatment. Expressed in general terms, the effect seems to be essentially *compensatory* in nature, and consists in restoring a physiologically balanced condition which has been disturbed by the first treatment. Thus if the initial action is cytolytic, the second is probably anti-cytolytic. In these concentrations the above anesthetics and cyanide have in fact a protective or anti-cytolytic action on eggs exposed to pure sodium chloride solution; and it seems thus probably that the favorable effects of the after-treatment depend on an action of essentially this nature—that is, consist in a modification of the plasma-membrane of the opposite kind to that produced by the primary or cytolytic treatment, in other words, in the direction of a *decrease* of permeability, or of an increase in the resistance to any permeability-increasing conditions that may be present.

Alcohols. After-treatment with alcohols gave results similar to the above, but on the whole more favorable. This increased favorability may however be due to the fact that most of the experiments with alcohols were performed during late July and August, at a time when the starfish eggs showed in general a greater responsiveness to parthenogenetic treatment than in June, in which month most of the experiments with the first group of anesthetics were made. During late July and early August eggs exposed to acid sea-water alone, without any after treatment, often yielded a considerable and in a few cases a high proportion of larvae. The effects of the after-treatment with alcohols are shown in table 6, in which are summarized the results of four series of experiments with both acetic and butyric acids, made on four successive days toward the end of July.

TABLE 6

In these experiments both acetic and butyric acids were used. The eggs were exposed, about one hour after removal, to sea-water containing 6 cc. n/10 fatty acid to 100 cc. sea-water, for one minute. After ten minutes they were brought into the solutions of the series where they remained thirty minutes; they were then returned to sea-water. Hypertonic sea-water and cyanide were also used for comparison. The figures give the approximate proportion of mature eggs forming free-swimming blastulae and gastrulae.

A. Acetic acid series

AFTER-TREATMENT	RESULTS			
	July 23	July 24	July 25	July 26
1. None (acetic acid alone)...	ca. 5 %	ca. 1 %	10-15 %	10-15 %
2. Hypertonic sea-water.....	no exp't.	ca. 3-4 %	ca. 10-15%	ca. 50 %
3. n/1000 KCN.....	30-40 %	ca. 50 %	ca. 90 %	ca. 70 %
4. 5 v. % ethyl alcohol.....	30-40 %	ca. 10 %	ca. 60 %	ca. 10 %
5. 2 v. % n-propyl alcohol...	ca. 20-25%	ca. 3-4 %	ca. 60 %	25-35 %
6. 0.8 v. % n-butyl alcohol...	ca. 35-40%	ca. 3-4 %	ca. 70 %	ca. 50 %
7. 0.25 v. % n-amyl alcohol..	ca. 25-30%	ca. 3-4 %	ca. 65-75%	ca. 40 %

B. Butyric acid series

1. None (butyric acid alone).	10-15 %	ca. 10 %	20-25 %	ca. 30 %
2. Hypertonic sea-water.....	no exp't	ca. 5 % (decrease)	30-40 %	ca. 50 %
3. m/1000 KCN.....	ca. 20 %	ca. 50 %	ca. 50 %	ca. 90 %
4. 5 v. ethyl alcohol.....	85-90 %	ca. 35-40%	5-10 % (decrease)	decrease (ca. 10 %)
5. 2 v. % n-propyl alcohol...	80-90 %	increase slight (<10 %)	40-50 %	decrease (10-15 %)
6. 0.8 v. % n-butyl alcohol...	ca. 60 %	ca. 10 %	ca. 50 %	ca. 50 %
7. 0.25 v. % n-amyl alcohol..	ca. 40 %	ca. 20 %	ca. 60 %	ca. 60 %

Controls. In all four series the great majority of sperm-fertilized eggs yielded normal larvae. The unfertilized eggs showed no development.

In three additional similar series with acetic acid, methyl alcohol (10 v. per cent and 8 v. per cent) and capryl alcohol, $C_8H_{17}OH$, (one-fifth saturated solution in sea-water) were used in addition to the above. Methyl alcohol proved unfavorable; capryl alcohol gave a well-marked increase in two of the experiments; in the third the acid treatment alone produced an unusually high proportion of larvae (70 to 80 per cent of the mature eggs) and all forms of after-treatment were unfavorable (cf. table 7).

This last phenomenon was observed in several series; those of July 25 and July 26, in table 6 (eggs treated with butyric acid), show it in part; two other series where a still higher proportion of eggs formed larvae after simple membrane-formation, illustrate this behavior more definitely. These series are summarized in table 7.

TABLE 7

The unfertilized eggs were treated for one minute with a solution of 6 cc. n/10 acetic acid plus 100 cc. sea-water, at about one-and-a-quarter hours after removal from the starfish. After ten minutes in normal sea-water they were exposed for thirty minutes to the solution, then returned to sea-water.

AFTER-TREATMENT	RESULT	
	July 6	July 30
1. None (acid treatment alone)	30-40 % of eggs form larvae	70-80 % form larvae
2. Hypertonic sea-water.....	No apparent increase	Slight decrease; 65-75 %
3. m/1000 KCN.....	Decrease; ca. 10 % larvae	Slight decrease; ca. 60 %
4. 5 and 6 v. % ethyl alcohol..	All dead next day	All dead next day
5. 2.5 v. % n-propyl alcohol...	All dead next day	All dead next day
6. 1 v. % n-butyl alcohol.....	All dead next day	All dead next day
7. 0.25 v. % n-amyl alcohol...	ca. 30 % larvae	A few larvae; ca. 1 %
8. 1/5 saturated capryl alcohol	No experiment	ca. 30-40 % larvae

Controls. Normal: almost all fertilized eggs from larvae. Unfertilized eggs undergo coagulation without membrane-formation or cleavage.

In these experiments the after-treatment not only effected no improvement, but on the contrary was definitely injurious. It would thus appear that the after-treatment is favorable only with those eggs which are unable otherwise to recover a normal condition. Eggs which are able spontaneously to recover from the effects of the initial cytolytic action are unfavorably affected by the subsequent anti-cytolytic treatment. As already suggested, this treatment appears to be essentially compensatory in its action; and merely aids in restoring what might be called a condition of physiological equilibrium. Hence, with eggs that spontaneously revert to the normal state after membrane-formation, the addition of the after-treatment is likely to result in over-com-

pensation and hence in injury. Whether the after-treatment is favorable or not thus depends on the physiological condition of the egg. Its action is at best supplementary, and is superfluous or injurious whenever the egg is capable of regaining the normal condition unaided.

As with the first series of anesthetics, it was found that treatment with alcohols alone, without preliminary membrane-formation, leaves the great majority of eggs apparently unchanged. A small and variable proportion of eggs so treated may, however, form membranes and develop, as in the above experiments with ether, urethane and chloretone. The essential physiological action of the alcohols, like that of the other anesthetics, is thus different from that of the membrane-forming agency, and probably consists in a modification of the membrane in the direction of decreased permeability.

Series III. Treatment with anesthetics after membrane-formation by temporary warming

Brief exposure of starfish eggs during the maturation-period to temperatures of 34° to 38° induces typical membrane-formation followed by cleavage, which in favorable instances leads to the production of a considerable proportion of larvae. As with the other membrane-forming agents, this proportion may be increased by after-treatment with hypertonic sea-water, cyanide, or anesthetics. Table 8 gives a summary of four typical series of experiments, in which urethane, chloretone, and ether show well-marked favorable action, while chloral hydrate and cyanide are less effective; hypertonic sea-water again proves relatively ineffective as compared with the other solutions. Two series of experiments with alcohols gave similar results, summarized in table 9.

After-treatment with alcohols is thus highly effective with eggs subjected to this type of membrane forming treatment. In these experiments (table 9) propyl, butyl and amyl alcohols appear more favorable than ethyl alcohol, and the same was very generally observed in last summer's experiments with starfish eggs. In my experiments with *Arenicola* larvae, propyl and butyl alcohols

TABLE 8

The eggs were placed at about the time of separation of the first polar body in sea-water at 35° for seventy seconds, and then returned to sea-water at ca. 20°. Ten minutes later they were placed in the following solutions where they remained thirty minutes; thence again to sea-water. The proportions of mature eggs forming larvae were approximately as follows:

AFTER-TREATMENT	PROPORTION OF EGGS FORMING LARVAE			
	June 19	June 20	June 21	June 24
1. None (warming alone).....	<5 %	5-10 %	ca. 5 %	ca. 5 %
2. Hypertonic sea-water.....	{ slight in-crease; ca. 5 %	{ no in-crease; 5-10 %	ca. 10 %	>5 %
3. n/1000 KCN.....	5-10 %	ca. 5 %	ca. 10 %	few: <5 %
4. 0.6 v. % ether.....	10-15 %	20-25 %	10-15 %	ca. 20 %
5. 0.1 % chloral hydrate.....	ca. 10 %	{ few; < ex-per. 1	{ no in-crease; ca. 5 %	few; ca. 5 %
6. 0.2 % urethane.....	no exp't	ca. 20 %	ca. 20 %	40-50 %
7. 1/10 saturated chloretone..	{ few; < ex-per. 1	ca. 25-30 %	ca. 25-30 %	30-40 %

Controls showed a good proportion of normal eggs; on June 21 many eggs failed to mature; on June 24 the proportion of fertilized eggs yielding larvae was smaller than usual.

Eggs from each lot were subjected to the same treatment after maturation was complete (3½ to 4 hours after removal); the results of these experiments were almost entirely negative. After maturation the eggs become relatively insensitive to this form of treatment.²⁹

TABLE 9

The eggs were warmed briefly to 35° as already described, and exposed for thirty minutes to the following solutions

AFTER-TREATMENT	PROPORTION OF LARVAE	
	July 27	July 29
1. None (warming only).....	ca. 10 %	2-3 %
2. Hypertonic sea-water.....	No increase; ca. 10 %	ca. 5 %
3. m/1000 KCN.	ca. 60 %	decrease; 1-2 %
4. 5 v. % ethyl alcohol.....	Slight increase: 10-15 %	15-20 %
5. 2 v. % n-propyl alcohol.....	50-60 %	20-25 %
6. 0.8 v. % n-butyl alcohol.....	75-85 %	15-20 %
7. 0.25 v. % n-amyl alcohol.....	50 %	15-20 %
8. 1/5 saturated capryl alcohol.....	No exp't	ca. 10 %

Both sets of controls were normal.

²⁹ Journal of Experimental Zoölogy, 1908, vol. 5, pp. 400 seq.

also showed a distinctly greater protective or anti-cytolytic action than ethyl alcohol.³⁰

It is remarkable that hypertonic sea-water, in the numerous experiments performed last summer, never proved more than moderately effective with starfish eggs, and in no case effected so great an improvement as occurred in favorable experiments with cyanide or anesthetics. With sea-urchin eggs, on the other hand, hypertonic sea-water of the above concentration gives highly constant and favorable results. It would seem that the action of hypertonic sea-water in artificial parthenogenesis is in no sense exceptional or distinctive, but that it is for mainly incidental reasons that this treatment proves so highly favorable with certain species of eggs, while with others a quite different form of treatment is more effective. Why such different agencies should produce the same physiological effects cannot at present be said in any detail. The common factor in the production of these effects is probably the presence of a membrane whose physiologically important properties, such as electrical polarization, are influenced by agencies of widely different kind in essentially the same manner. The case of stimulation again affords perhaps the best analogy; here polarization-changes at membranes are demonstrably concerned, and we find that a large variety of stimuli may cause the same effect. The possibilities of variation in polarization, *qua* polarization, are limited; a given agency may either decrease the polarization of a membrane or increase it, and the degree, rate, and periodicity of these changes may vary. What is significant is that the number of variables is constant and limited. It is thus not surprising that a variety of external changes should produce the same physiological effect, if this effect depends on a change of polarization. The phenomena under consideration in the present paper appear to illustrate this principle.

³⁰ Cf. American Journal of Physiology, 1913, pp. 264 seq.

III. SUMMARY

The chief experimental results and theoretical conclusions of the present paper may be briefly summarized as follows.

1. Temporary exposure of unfertilized starfish eggs to pure isotonic NaCl solution, to sea-water containing fatty acid, or to high temperature (35°), is followed by the formation of fertilization-membranes and cleavage, and a certain proportion of eggs so treated (usually from 1 to 5 per cent) may develop to a larval stage. If, after membranes are formed, the eggs are treated for thirty minutes with hypertonic sea-water or weak cyanide solution, the proportion thus developing is usually increased, often to a marked degree.

2. The same effect is produced in *Asterias* eggs by after-treatment with solutions of various anesthetics in sea-water, namely, ethyl ether, ethyl urethane, chloral hydrate, chloretone, various alcohols (ethyl, propyl, butyl, amyl, capryl). *Arbacia* eggs do not respond to this treatment.

3. The anesthetics exert this favorable action in concentrations corresponding closely to those causing typical anesthesia in *Arenicola* larvae. The essential effect of this treatment upon the egg is thus probably of the same nature as that which conditions anesthesia in irritable tissues. This effect appears to consist in imparting to the plasma membranes either a degree of permeability which is *less* than the normal, or an increased resistance to agencies whose general action is permeability-increasing (that is, cytolytic, if the action is not compensated or reversed within a certain time).

4. The above anesthetics have been shown to exert a protective or anticytolytic action on starfish eggs in the presence of certain cytolytic agents (pure salt-solutions). Their action in preventing the disintegration of eggs after artificial membrane-formation, and in thus rendering possible the continuance of development, is probably an effect of the same essential kind.

5. The view is put forward that the essential physiological effects produced by the two successive treatments in artificial parthenogenesis are opposite in character and correspond respectively to the depolarizing and the repolarizing phases of the stimulation-process in irritable tissues in general. The primary or membrane-forming treatment has a permeability-increasing and hence depolarizing effect upon the plasma membrane. This initial depolarization is probably the critical or determinative event in fertilization, as well as in stimulation. A return of the plasma membrane to or toward the original semi-permeable and electrically polarized condition within a certain time (*ca.* fifteen minutes at 20°), is, however, essential if normal development is to follow, otherwise cytolysis results. This recovery of the normal semi-permeability of the membrane, with the correlative electrical polarization, is favored by after-treatment with agencies (cold, cyanide, anesthetics, hypertonic sea-water) whose general action is permeability-decreasing or anti-cytolytic. Hence, such after-treatment increases the proportion of eggs that regain their normal properties and continue development.

A COMPARATIVE STUDY OF THE CHROMOSOMES IN ORTHOPTERAN SPERMATOGENESIS

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TEN PLATES

CONTENTS

I. Introduction.....	652
II. The spermatogonial chromosomes.....	655
a. Hippiscus type.....	655
b. Stenobothrus type.....	655
c. Comparisons.....	657
1. Acrididae.....	657
2. Locustidae.....	659
3. Gryllidae.....	660
4. Phasmidae.....	660
5. Blattidae.....	660
6. Forficulidae.....	661
d. Summary of results on spermatogonia.....	661
III. Synapsis period.....	662
IV. Chromosomes of the first spermatocyte prophase.....	662
a. Evidence for chromosome individuality.....	662
1. Chromosome reproduction.....	663
2. Constancy of complex in species, etc.....	663
3. Definite relation to archoplasmic fibers.....	663
4. Individual peculiarities of behavior.....	664
b. Relation of the first spermatocyte chromosomes to those of the spermatogonium.....	665
1. Accessory chromosome.....	665
2. Euchromosomes.....	665
c. Forms of prophase tetrads.....	667
V. Chromosomes of first spermatocyte metaphase.....	670
a. Identification of metaphase chromosomes of the first spermatocyte with those of prophase.....	670
b. Movements of chromatids in metaphase.....	671
c. Relation of chromosomes to archoplasmic fibers.....	674
1. Hippiscus type.....	674
2. Stenobothrus type.....	675
VI. Chromosomes of second spermatocyte metaphase.....	678

VII. Discussion of results on the spermatocytes.....	679
a. Acrididae	680
b. Locustidae	689
c. Gryllidae	691
d. Blattidae	692
e. Phasmidae	695
f. Forficulidae	695
VIII. Conclusions.....	696
a. Relation between chromosomes of the first spermatocyte prophase and those of spermatogonium	696
b. Composition of chromosomes in first spermatocyte prophase.....	700
1. Rod-shaped tetrads.....	701
2. V-shaped tetrads.....	702
3. Cross-shaped tetrads.....	703
4. Ring-shaped tetrads.....	703
c. Relation of chromosomes of first spermatocyte metaphase to those of the prophase	707
d. History of the tetrads in first spermatocyte mitosis.....	707
1. Rod-shaped tetrads.....	707
2. V-shaped tetrads.....	708
3. Cross-shaped tetrads.....	708
4. Ring-shaped tetrads.....	710
Hippiscus type.....	710
Stenobothrus type.....	716
e. The chromosomes in the second spermatocyte.....	718
1. Hippiscus type.....	719
2. Stenobothrus type.....	720
List of Orthoptera whose spermatogenesis has been described.....	723
Bibliography of Orthopteran spermatogenesis	725

INTRODUCTION

Since the beginning of investigations into the phenomena of the maturation processes there have been conflicting views regarding the nature of the two mitoses which so closely follow each other without the customary resting stage intervening. Almost from the first this period in the history of the germ cells has been assigned great importance and a necessity for a thorough understanding of the facts has been realized. Among those possessed of an intimate knowledge of the complicated changes here taking place there has come to be a general agreement that of all the cell parts the chromatin gives every evidence of being primarily important. Along with this has arisen the conviction

that its organization is very precise and that the most tangible evidence of this is its method of integration into a definite and characteristic series of units in each kind of organism.

From many careful studies of a great number of different animals and plants the following facts have been determined beyond any reasonable doubt: 1. In both male and female—with a significant exception—there occur identical and duplicate series of these chromatin units. 2. During maturation this duplicate series is reduced in number to one-half by the joining of homologous pairs together. 3. In the course of the two maturation mitoses these paired elements are disjoined with the final result that each mature germ cell is provided with one representative of each pair. 4. When the paternal and maternal germ cells thus produced come together in fertilization there is restored again the duplicate series with which the process started.

There is nothing to indicate that any other parts of the cell have such significance as the behavior of these chromatin units indicates is theirs, and accordingly the phenomena of their junction and disjunction requires the most exact analysis. There are many complicated changes taking place here, and the conditions are such as to make it difficult, or even impossible in some cases, to determine exactly what are the facts. Each step in the process must however be understood and no effort should be spared to ascertain all the facts. Of the greatest importance are the stages immediately following the last division with the unreduced number of chromosomes. At this time, undoubtedly, changes of vital significance occur, but unfortunately they are very involved and difficult to determine in detail. However, one fact, which is the important one in connection with the present study, stands out clearly: the series of chromosomes present in the last mitosis with the unreduced number reappears in easily recognizable form in the prophase of the first maturation division. The only difference of importance is that instead of being present in a series of single elements, the chromosomes are now joined together in pairs so that the number of independent chromatin bodies is one-half of that in the preceding generation. But noth-

ing has been lost from the cell and, so far as the morphological identity of the chromosomes is concerned, no difference exists except their pairwise union.

The important phenomena of the synapsis stage will receive consideration in later papers by my students and myself. At the present time I wish merely to consider the comparatively simple conditions apparent in the late prophase of the first maturation division and in the succeeding mitoses. This is an important matter because from studies of these stages, and upon practically identical material, all the possible interpretations of the maturation divisions have been advanced. Diversity of opinion regarding maturation phenomena in unlike materials may therefore be due, as in this case, not to differences in fact but merely to variety of interpretation. Also, regardless of the exact nature of the synaptic changes it is of great importance to know just what takes place in the maturation mitoses, for often a correct interpretation of the prophase chromosomes is followed by a faulty account of their actual division later. No matter how accurately the processes involved in the formation of the tetrads is understood, there could be no solution of the problem of chromosome distribution in the maturation divisions so long as the present uncertainties regarding their movements continue. I therefore desire, in this paper, to discuss the actual movements of the chromatids, regarding it as determined that in the tetrads we have a fusion of homologous chromosome pairs longitudinally divided.

I shall attempt therefore a comparative presentation of the conditions during these later phases of the process, and shall venture a critical consideration of the papers of other authors on the same subject in the hope that a consensus of opinion may be reached. I venture to hope also that my long study of this subject, my extensive comparative acquaintance with Orthopteran material and the consistent results of my students may be taken into consideration in judging my effort to arrive at some conclusion in these disputed matters.

THE SPERMATOGONIAL CHROMOSOMES

a. Hippiscus type

It is aside from the purpose of the present paper to enter far into a discussion of conditions in this generation of the germ cells. In order that the facts may be before us it will however be necessary to state briefly what is known about the chromosomes at this time. For details reference should be had to Sutton's ('00) paper. A polar view of a spermatogonial metaphase shows 23 elongated, rod-shaped chromosomes, arranged radially about the spindle axis with fiber attachment at the inner end (figs. 20, 44, 77, 94, 98). In the anaphase the chromosomes separate, beginning at the *inner* end, and move to the poles of the spindle as simple straight rods. Later they lie side by side, approximately parallel, and become vesicular, with the chromatin on the periphery. During the prophase of the succeeding mitosis the chromatin contracts to the center of each vesicle and the definitive chromosomes of the late prophase reappear in the same relative positions they occupied in the preceding telophase. The cells of one young Chortophaga nymph showed an interesting modification of this condition. The largest four pairs, instead of being separate, were joined together at their ends and appeared in the form of *tetrads* with the fiber attachment at the point of junction (fig. 94). In the anaphase each chromosome commenced to divide, as usual, at this point and in these cases the anaphase chromosomes were V-shaped with the apex (synaptic end) directed towards the pole of the spindle. All the cells of this individual showed the same conditions.

b. Stenobothrus type

Exceptions to many general conditions are found in the appearances presented by the cells of *Stenobothrus*. This is a genus that has been extensively studied and, in general, correctly interpreted. Because of its exceptional nature many generalizations based upon it are wrong. This will appear clearly as the discussion proceeds. To avoid confusion I shall, throughout this

paper, consider *Stenobothrus* separately and it will be excluded from general statements except as indicated otherwise.

The spermatogonia of *Stenobothrus* show two important exceptions to the general statement already made. These are (1) the number of chromosomes is 21 instead of 23 (17 in some species, reported by others); (2) the point of fiber attachment may be terminal, subterminal or median instead of always terminal. For this latter reason the chromosomes are not always rod-shaped but may also be V-shaped or J-shaped. The important thing to note here is that the point of fiber attachment is a definite part of the organization of the chromosomes and is *constant*, not only for the cells of this generation but also for those of the first and second spermatocyte. I have material from only one individual of this species,¹ with 21 chromosomes, and in spermatogonia of this there are always 12 chromosomes with terminal fiber attachment and 9 with subterminal and median. One of these with median attachment is the accessory chromosome, so that there are four pairs with non-terminal fiber connections. The exact point at which this relation to the fibers is established may vary somewhat, but as may be seen in figures 59, 60, 61 and 62 it is easy to distinguish these elements from the ones with terminal attachment. What relation there may be between the difference in number of chromosomes and the shifting of fiber attachment I do not know, but the thought at once suggests itself that this may be due to an altered cross segmentation of a continuous spireme thread which, if there were continued chromosome individuality, would produce multiple chromosomes. Whatever may be the cause it is fortunate for our study that it operates, because it gives us a variation from the typical conditions in the group which is very helpful in coming to an understanding of the movements of the chromosomes in the maturation divisions.

¹ I am not sure of the identification of this material. It was among a large collection of specimens put up for me by a student on an early expedition into western Kansas, and the specimen from which it came was not preserved. At the time I made my first study of it no other form but *Stenobothrus* had been reported with such chromosomes, but since then I have found them also in *Chorthippus* and *Chloëaltis* and Miss Carothers in some species of *Trimerotropis*. For this reason I can refer it to no genus with certainty.

c. Comparisons

1. *Acrididae*. The points of interest for this study regarding the chromosome condition in the spermatogonia concern (1) the number of chromosomes, (2) their sizes and forms, (3) the positions of their fiber attachments, (4) their arrangement in the metaphase plate and (5) their behavior in mitosis. I will briefly give the results of other investigators and then compare them with my own.

Buchner ('09) reports 23 chromosomes in *Oedipoda*, all rod-shaped, with terminal fiber attachment, and arranged in the typical way. He is however unable to recognize homologous pairs. Brunelli ('10) in *Tryxalis* finds 23 rod-like, radially arranged chromosomes of various sizes with terminal fiber attachments, but no recognizable pairs. Davis ('08), from studies on *Dissosteira*, *Arphia*, *Hippiscus*, *Chortophaga* and *Melanoplus*, distinguishes the 23 typical chromosomes showing the paired condition, radial arrangement and terminal fiber attachment. Granata ('10), in *Pamphagus*, is able to find only 19 chromosomes, all of which are typically Acridian in their form and behavior. Montgomery ('05) is the only investigator who reports an even number of chromosomes in any member of this family, and, in addition, a variation of the number within the genus. This number is 20 or 24 in *Syrbula acuticornis*. In other respects Montgomery's results are typical for the *Acrididae*, but the even number requires the accessory chromosome to be bivalent. My own students have invariably reached the same general conclusions regarding the nature and behavior of the spermatogonial chromosomes. Thus, Carothers in *Arphia*, *Brachystola* and *Trimerotropis*; Hartman in *Schistocerca*; Nowlin in *Melanoplus*; Pinney in *Phrynotettix*; Sutton in *Brachystola* and Wenrich in *Phrynotettix* find typical Acridian conditions throughout. Robertson ('08) also, in *Syrbula admirabilis*, after a very careful study, is quite unable to agree with Montgomery regarding the number of chromosomes and the paired condition of the accessory chromosome, and finds the chromosome behavior in this genus typical of the group.

The conditions in *Stenobothrus* have been reported with much uniformity by Davis ('08), Gerard ('09), Meek ('12) and de Sinéty ('01). Davis and Meek find definitely 17 chromosomes and Gerard suggests the same as his best enumeration. De Sinéty does not give definite evidence on this point. According to Davis the largest 3 pairs are bent rods and have non-terminal fiber attachments at the points of curvature; the remaining 5 pairs are normal rod-shaped elements with terminal attachments. In these matters Meek agrees, and, while the descriptions of Gerard and de Sinéty are not definite and specific, their general statements and figures substantiate Davis' findings. The accessory chromosome is not unusual in its behavior.

My own studies have extended over more than forty genera of Acrididae as follows: *Acrolophitus*, *Aeoloplus*, *Amphitornus*, *Arphia*, *Aulocara*, *Boopedon*, *Brachystola*, *Camnula*, *Chloëaltis*, *Clinocephalus*, *Dactylotum*, *Dissosteira*, *Encoptolophus*, *Eremnus*, *Hadrotettix*, *Hesperotettix*, *Hippiscus*, *Mecostethus*, *Melanoplus*, *Mermiria*, *Mestobregma*, *Orphulella*, *Paroxya*, *Philbostroma*, *Phrynotettix*, *Phaetaliotes*, *Proracorypha*, *Pseudopomala*, *Psinidia*, *Rhomaleum*, *Schistocerca*, *Scirtettica*, *Spharagemon*, *Stenobothrus*, *Syrbula*, *Trimerotropis*, *Tropidolophus*, *Tryxalis*. In all these the same conditions were found as have been described for *Hippiscus* regarding the number of chromosomes, their sizes and forms, the attachment of the fibers, arrangement in the metaphase and behavior during division, with the exception of *Stenobothrus*, *Chorthippus*, *Mermiria*, *Hesperotettix*, *Chloëaltis*, some species of *Trimerotropis* and, occasionally, *Chortophaga*. In *Stenobothrus* there is the same apparent difference in number, shifting of fiber attachment in certain chromosomes and difference in form and behavior in these elements as have been described by other investigators. *Hesperotettix* and *Mermiria* have the association between the accessory chromosome and certain of the euchromosomes that I have elsewhere described ('05) which produces an apparent change in the behavior of the complex, while a certain individual of *Chortophaga* in the early nymph condition showed a spermatogonial synapsis of 4 pairs of chromosomes.

It would seem very evident therefore that in the spermatogonia of the Acrididae we are dealing with a chromosome complex of a very definite and precise organization which, in the great majority of cases, presents itself without essential variation in number, size and form, fiber attachment, arrangement in the metaphase and behavior during division of its elements. *Stenobothrus* and *Pamphagus* seem to be definite exceptions in some of these respects, but doubtless were we more familiar with all the facts in the case a larger harmony would become manifest.

2. *Locustidae*. I desire to consider with much detail only the conditions in the Acrididae, but since exceptions to the general behavior of the chromosomes in the Orthoptera have been taken from studies on other families I shall be obliged to give them consideration. This I shall do as briefly as possible, going into details only when it is necessary.

My own work began on the *Locustidae* and I made a comparative study of *Xiphidium*, *Anabrus*, *Orchesticus*, *Scudderia*, *Conocephalus* and some others which I did not identify. So far as I was able to go at the time there seemed to be a uniformity here also, but of a somewhat different order. Instead of 23 chromosomes there appeared 33 and the accessory chromosome was very large and conspicuous. The chromosomes were rod-shaped, arranged radially in the metaphase with terminal fiber attachments, and they divided regularly into two equivalent straight rods by a cleft extending from the center outward in the plane of the equatorial plate—all except one chromosome in *Anabrus* which at the time I could not explain, but later recognized as a multiple. None of my students have worked on this family but other investigators have, and the result has been less uniformity in chromosome numbers than in the Acrididae but in other respects no essential differences. Thus Stevens ('05) reported 46 chromosomes in *Stenopelmatus*, but later recognized this as an error and gave the correct count as 47. In *Ceuthophilus* ('12) she found 37 chromosomes but in both cases no exceptional behavior. Davis ('08) and Meek ('12 a) in *Steiroxys* agree on 29 as the number present with no atypical behavior. De Sinéty ('01) studied *Decticus*, *Platycleis* and *Orphania* but gives the

chromosome number for only the latter where he found 31. Buchner ('09) however also gives 31 for *Decticus*, while Vejdvský ('12) reports but 23 for the same species. Taking these results at their face value it is evident that in the *Locustidae* there is an unequal diploid chromosome group with an unpaired accessory chromosome and all the other characters that have been considered in connection with the *Acrididae*.

3. *Gryllidae*. Careful detailed studies of the chromosome relations in the *Gryllidae* are few. Baumgartner ('04) reports 29 chromosomes in *Gryllus assimilis* and 21 in *G. domesticus*. This latter number was also found in the same species by Guthertz ('07) and in *G. desertus* by Brunelli ('09). Buchner ('09) and Voinov ('04) have both studied *G. campestris* but without giving any results of value on chromosome relations. *Gryllotalpa* has been studied by Buchner, Payne, vom Rath and Voinov. Vom Rath ('91) reported at length on the spermatogonia of this animal and his figures were widely copied, but it is very evident that he was fundamentally in error regarding the most essential part of his work. He announces 12 as the diploid chromosome number but the internal evidence and the results of other investigators show that this is really the haploid condition.

4. *Phasmidae*. Studies on the *Phasmidae* are not numerous. Practically the only ones from a modern standpoint are those of de Sinéty and Jordan and in the former case the work is lacking in needed detail. De Sinéty finds 36 chromosomes in *Leptynia* with a typical accessory chromosome joined to a tetrad in the spermatocyte. If this be counted a separate chromosome for each univalent part there is an odd number. Jordan ('08) seems to determine definitely the number 35 in *Aplopus*. Since de Sinéty's enumerations are evidently not made with great care it is possible that in *Leptynia* the same number is present. *Dixipus* and *Menexenus* were also studied by the French investigator but no chromosome counts are given.

5. *Blattidae*. Of the cursorial *Orthoptera* the *Blattidae* have been most studied. Morse ('09) investigated four genera and found in *Blatta*, *Leucophaea* and *Stylopyga* a diploid group of 23 paired elements of typical *Orthopteran* behavior, while in *Peri-*

planeta there were 33. *Blatta* has also been studied by Stevens ('05) and Wassilieff ('07) with the same numerical results. Farmer and Moore ('05) report 32 chromosomes for *Periplaneta* plus a conspicuous nucleolus which the authors believe is lost during each mitosis. The work of the other students of the Blattidae makes it certain however that the English investigators incorrectly interpreted the behavior of the accessory chromosome which is characteristically Orthopteran in this family.

6. *Forficulidae*. The Forficulidae, taxonomically, are not now regarded as a family of the Orthoptera and their germ cells show a characteristic difference in the nature of the accessory chromosome. According to Randolph ('08) in *Anisolabis*; and Stevens, de Sinéty, Zweiger, and possibly St. George, in *Forficula*, there is an even number of chromosomes, 24, in the spermatogonia. Of these, according to Stevens, one pair constitutes an unequal tetrad in the first spermatocyte and has all the characters of an idiochromosome pair of the Hemiptera and Coleoptera.

d. Summary of results on spermatogonia of Orthoptera

From the foregoing statements I believe the following conclusions are justified: (1) The diploid number of chromosomes in the Orthopteran male is uneven; (2) The diploid complex in each case may be resolved into a duplicate series characterized by differences in relative size; (3) an odd element, sometimes distinguishable by excessive size, may not thus be grouped with a mate and is further marked by occasional peculiarities of behavior; (4) the typical form of the spermatogonial chromosome is a rod which takes a radial position in the equatorial plate of the metaphase with the fiber attached at the inner end; (5) in division the longitudinal halves of the chromosome separate in the plane of the equatorial plate, beginning at the inner end, and pass to the pole as straight rods; (6) an exception to (4) and (5) is furnished by certain chromosomes of *Stenobothrus*, *Chorthippus*, *Chloëaltis* and *Trimerotropis* which are J- or V-shaped with the fiber attachment at the bend.

SYNAPSIS PERIOD

As has already been stated, we have no exact knowledge of the changes taking place at this time. Their significance is unquestionably great, and no pains should be spared to determine their character fully. But even were they completely understood we should still be lacking a continuous picture of well defined, individual chromosomes throughout their entire history for, succeeding the synaptic phase, comes a period when the chromosomes become very diffuse and their outlines with difficulty distinguishable. We may not therefore hope to be able to recognize the chromosomes in a compact and sharply defined form through the various stages of their development. That they are however morphologically continuous seems to me unquestionably evidenced by the fact that, succeeding all these involved and complicated changes, there is finally a reappearance of the same series of elements in the same cell, with the one difference that instead of twenty-three single chromosomes in the Acrididae there are now eleven pairs, plus one chromosome that continues on unchanged. I believe, therefore, that we have every reason to consider that in the prophase of the first spermatocyte we are dealing with the same chromosomes that came into the cell when it was produced by the division of the last spermatogonium. Whether union was made side by side or end to end in the synaptic period has not been determined, but it is very clear that in the last prophase the chromosomes show undoubted telosynapsis.

CHROMOSOMES OF THE FIRST SPERMATOCYTE PROPHASE

a. Evidence for chromosome individuality

Fundamental to the entire conception of chromosome relations is the belief that we are dealing with persistent morphological entities. If this be not true then the whole question of the constitution of the chromosomes and the method of their division is of no theoretical importance. That they are of this definite and significant character is however evidenced in many ways. In

a later paper I hope to enter into this matter more fully, but for the present I will merely indicate the facts relating to Orthopteran spermatogenesis which support this view.

(1) There is the fact of greatest significance that in each cell division they perpetuate themselves, and by a process suggestive of the utmost accuracy and importance. The means for conserving the continuity of each chromosome is therefore clear. (2) The result of the operation of this process is demonstrated by the observation that not only does the individual animal show in each of its cells the same series of elements but all the members of the species, genus and even family, with few exceptions, have it. The observed process and the results of its operation through countless cell generations is thus demonstrated. Accordingly, any one chromosome has the form, size and behavior which characterize it for the reason that it is the direct material descendent of a line, each member of which possessed these same characteristics.

It ought not to be necessary, in writing for biological readers, to say that in making such a statement I do not in any sense impute omnipotence, omniscience or immutability to the chromosomes. But the absurd lengths to which some opponents of chromosome investigations have gone in attempting to break down the facts regarding them perhaps make it desirable for me to add that I have always considered the chromosomes as a part of the organic mechanism, reacting to each other and to their environment, as all living substance must do in order to exist. They neither make the organism nor are they made by it, but they do perpetuate themselves and perform functions of vital importance to each cell and to the entire organism. My position in this matter is more fully outlined in a former paper ('08).

In addition to these two facts regarding the reproduction of the chromosomes there are others which speak strongly for their individuality. These concern questions of organization and behavior and will be referred to more at length throughout the paper. At this point I will briefly mention them: (3) Constancy in relation to the spindle fibers. In almost every case the attach-

ment in the spermatogonia is at the end of the chromosome and when they join in pairs these ends are in contact, so that in the first spermatocyte the tetrads have the form of V's with the same orientation to the spindle as in the previous generation (figs. 32-58). In *Stenobothrus* however there are median and subterminal attachments and *these are the same in both generations of spermatocytes as in the spermatogonia* (figs. 59-76).

(4) Occasionally there are peculiarities of behavior which are constant and uniform. In *Hesperotettix* there is a union between the accessory chromosome and one of the tetrads. The members of this combination are always identical and their union never fails. Moreover in different species the proportionate size of the parts varies in conformity with the structure of the body. Thus figure 79 shows the condition in *H. speciosus*, figure 80 in *H. viridis* and figure 81 in *H. pratensis*. In *Brachystola*, as Miss Carothers has discovered, there is a pair, the members of which are unequal, and this is a constant condition accompanying their separation in the first spermatocyte. In *Phrynotettix* Mr. D. H. Wenrich has found an unequal pair also, one member of which is distinguishable from the other by its granular condition in the metaphase. In this case however the reduction division occurs in the second spermatocyte. This element in the first spermatocyte is shown in figures 88 to 91 and will be described in detail by Mr. Wenrich.

Supporting in every way these observations upon Orthopteran cells is the consistent background of similar facts in other groups of animal and plants and the significant parallel between their behavior and character development in breeding experiments. The persistence of individual chromosomes in multiple complexes of giant cells and heterospermized eggs is conclusive evidence of their real individuality and continuity. There are many other facts of this character to demonstrate the individuality and morphological continuity of the chromosome, but for the present purpose these will serve to show the foundation for my belief regarding them.

b. Relation of first spermatocyte chromosomes to those of the spermatogonium

(1) If then we are dealing with a morphologically continuous series we must recognize in the twelve chromosomes of the first spermatocyte the twenty-three chromosomes of the last spermatogonial division. Regarding one of these, the accessory chromosome, there can be no question. After the last spermatogonial division it removes itself from the others, often into a separate vesicle, and, during all the complicated changes of the synaptic period, may be recognized as a separate and distinct structure. It does not divide in the first spermatocyte but, in the form characterizing it in the spermatogonial metaphase, it passes to one pole of the spindle and is included in but one of the two daughter cells (figs. 36 and 45). In the second spermatocyte, showing the same form as in the two preceding generations, it takes its place in the equatorial plate and is divided like its fellows. In this one case the chromosome may be traced with little change through the whole of this period.

(2) There remain to be considered the twenty-two other chromosomes. It may be seen in the spermatogonial metaphase that these are not of the same size but form a series, the largest of which is many times the length of the smallest. When these are studied carefully it is found that for each size there are two representatives (figs. 44 and 98). In the first spermatocyte there are eleven chromosomes, each composed of two approximately equal halves, the point of contact being in the middle of the length of the elongated rod. If the chromosomes are morphologically continuous there is no other conclusion possible than that we have here the twenty-two spermatogonial chromosomes united into eleven pairs. Still more conclusive on this point are the conditions in *Stenobothrus* where not only the duplicate series of chromosome sizes appears, but where, because of variations in fiber attachment, there is found a similar duplication according to form.

It is probably true that until the relations of the chromosomes during the synaptic phase are definitely determined it will not

be possible to assert unequivocally that the longitudinal axes of the paired chromosomes of the first spermatocyte represents the coincident axes of the spermatogonial chromosomes constituting it, for if there be a parasynapsis at any period there is a possibility that the doubly split thread may open out along the plane of the equational cleavage instead of along the space between chromosomes. In either event the form of the resulting chromosome would be the same. There are however some criteria for judging such relations and these, taken in consideration with the movements of the chromosomes in the spermatogonial divisions, make it appear highly probable that the evident telosynapsis of the late prophase actually exists as such.

Two observations strongly support this conclusion. There is first the case of the unequal tetrad, where two spermatogonial chromosomes of unlike size are joined together end to end. If the chromosomes are persistent individuals there can be no questioning this relation. Again, in the multiple chromosome, we have established the coincidence of the longitudinal cleft of the accessory chromosome with that of the tetrad to which it is joined. Because the accessory chromosome is a simple spermatogonial element its plane of cleavage is determined beyond doubt, and since in the spermatogonia it is joined end to end with one-half of the tetrad, the latter's cleft is identified. The remaining member must, of necessity, be joined by its end to its mate and so continue its division plane with that of the other members of the multiple. An additional, though less obvious piece of evidence, is furnished by a comparison between the chromosomes, especially the annular ones, of *Hippiscus* and *Stenobothrus*. It seems evident that there are here two types of division for certain chromosomes, provided the earlier history of each is the same. All the structural relations are similar in these two cases except the position of the fiber attachment and it is difficult to believe that their early history is different. If they are similarly constituted then the presumption is strong that those which lie with their length in the equatorial plate of the first spermatocyte are divided along their longitudinal cleft while those placed in the axis of the spindle with fiber attachment midway between

the ends of the univalent components would then suffer a reduction division.

To this comparatively direct observational evidence must be added the strong presumptive proof that the sister halves of a chromosome remain closely united here as in other generations of cells, and that separation between the parts of a tetrad is much more likely to occur along spaces between whole chromosomes. If this were not true, and if the annular space of the ring chromosome and the angle of the V-shaped elements should be the longitudinal cleft, then there exists a fundamental alteration in the composition of the chromosome and a wide diversity in the behavior of daughter chromatids not only in different species but also in the cells of one organism. From all these considerations it therefore appears highly probable that the rod-shaped tetrad of the late prophase and metaphase of the first spermatocyte represents two spermatogonial chromosomes joined end to end with their longitudinal clefts continuous. We have therefore to determine the relations of the various forms of the first spermatocyte prophase chromosomes to this typical element, and then to trace these into the metaphase of this division, and through the anaphase, in order to establish the fate of the parts of the tetrads.

c. Forms of prophase tetrads

There is apparently a great diversity of form in the prophase chromosomes, but while this is true, so far as external configuration is concerned, in the fundamental matter of organization the rule is uniformity. In discussing this organization certain elements and relations must be regarded. They may be summarized as follows: number of parts—four chromatids; relationship of parts—one pair of maternal, one pair of paternal chromatids, each pair produced by the longitudinal division of a mother chromosome, all four homologous; spatial relation of parts—daughter chromatids more or less in contact along the plane by which they were produced from the mother element, homologous chromatids united endwise; attachment of spindle fiber—at the same point as in previous generations, as a rule at the point where

homologous chromosomes are joined together, but occasionally at the opposite end or between the extremities. All the features enumerated are constants except the one involving the spatial relations, and to the variations here occurring are due the diverse forms of chromosomes found in the first spermatocyte. The problem is therefore to determine how it is possible to derive from a chromosome consisting of four similar chromatids, with constant and uniform fiber insertion, the various rods, rings, crosses, V's, double V's, K's, X's, U's, I's, 8's, and other less common forms, without alteration of structure and merely by change of the spatial relations of the four elements.

A written description of these permutations is not easy, but it is a very simple matter with a clay model to produce every form to be found merely by changing the relative positions of its four parts. These changes fall into three general types of movement: (1) flexures in the plane of the longitudinal cleft, (2) movements of the chromatids in a plane normal to that of the longitudinal cleft, (3) divergence of the four chromatids at the center while the ends remain in contact. In addition to these simple movements there are (a) combinations of these and (b) rearrangements of the four chromatids resulting in various complicated forms. As mitosis proceeds these combinations, especially of the first (a) kind, become more common and in the metaphase and anaphase, the rule. The point at which movement occurs in classes (1) and (2) is at the center of the rod where homologous chromosomes are joined together, and this fact cannot be devoid of significance.

The simplest relations are those found in the rod-shaped chromosome where the four parts lie extended along one axis. These I-shaped tetrads are commonly the smaller ones of the complex and do not often occur without some enlargement at their middle where the homologous chromosomes are fused. They represent the final step in the divergence of the joined parental chromosomes which, if there be an earlier parasynapsis, finds its opposite in the side to side position of these units (fig. 117).

Related to this simple form are those of the first category produced by flexure in the plane of the longitudinal cleft, and which

may be grouped into those having free ends and into those with the ends joined. In most species the majority of the chromosomes belong to the first of these types, but in some cases more of them are joined terminally. With but few exceptions the members of any complex will be found included in these two categories of the first group. As a result of these flexures the chromosomes have the form of V's, U's, incomplete rings and complete rings. There is no essential difference between the V-shaped chromosome and the annular form except the extent to which the bending has taken place, or, if there was a previous parasynapsis, the degree of separation of the ends. Movement of the chromatids in a plane normal to the plane of the longitudinal cleft results in the formation of various cruciform tetrads. These changes are likely to occur in the smaller members of the complex and result in the gradual separation of daughter chromatids and the approximation of homologous derivatives. If carried to the extreme the final result is the production of the same I-shaped chromosome as that present at the beginning of the process but with a transposition of the two axes, the shorter, transverse one coinciding with the original longitudinal cleft of the two homologous chromosomes.

Commonly there are flexures of the longer arms of the cross producing a variety of forms depending in part upon the point of view. When placed obliquely the cross may appear X- or K-shaped. Divergence of the four chromatids, except at the ends, constituting the third class of movements, produces the double V-shaped element which appears under a variety of forms when viewed from different angles. One of the included spaces represents the longitudinal cleft of the homologous chromosomes, now bent at an acute angle to each other; the remaining one, lying in a plane at right angles to the first, is the space between the homologous conjugants. It seems impossible to distinguish these apart in most cases. Such a separation of the chromatids is only temporary and a double-V chromosome is never seen in the metaphase. All the tetrad forms are most clearly differentiated in the late prophase, just before going over into the homogeneous condition of the metaphase, for at this time there is less of irregu-

larity in direction and contour of the parts. Nevertheless the various forms of this late period may be traced back by gradual steps into the earliest stages of the prophase where all the chromatin elements are in the form of very elongated, delicate threads such as are shown in figures 99, 101 and 105. These stages are best seen in smear preparations in which the nuclear elements are more isolated and where their parts are more distinct. There can be no doubt of the composition and movements of these tetrads and the only uncertainty relates to the early stages where, for a time, it is impossible to trace the individual chromatids. Whether telosynapsis alone, or parasynapsis in connection with it, is the history of chromosome association has not yet been definitely determined, but however this may be, it is certain that the four parts of each chromosome are derived by the longitudinal splitting of two homologous spermatogonial chromosomes.

CHROMOSOMES OF FIRST SPERMATOCYTE METAPHASE

a. Identification of first spermatocyte metaphase chromosomes with those of the prophase

At the end of the prophase the forms of the chromosomes have been established and, upon the dissolution of the karyotheca, they lose their granular character and become homogeneous. Aside from this they suffer no important change and all the forms seen in the prophase, with the exception of those resulting from wide separation of the chromatids at the middle of the tetrad, may be traced into the equatorial plate. Since however there have been extensive and continued movements of the chromatids with relation to each other, it is only reasonable to expect their continuance during the metaphase, and this, in fact, occurs. Regarding however the late prophase and early metaphase it may be stated that the chromosome forms of the early condition may be traced directly into the later, and what changes there are may be ascribed to a continuation of processes already in operation. It is essential to note this, for by some the bipolar condition of the cell is considered to inaugurate entirely new conditions and the separa-

tion of the chromatids to follow from these changed circumstances. While there may be a period of equilibrium established at the metaphase the character of the chromosomal movements does not materially alter.

b. Movements of chromatids in metaphase

The metaphase movements of the chromosomes have given rise to the most contradictory accounts, due often to the lack of an exact acquaintance with the real structure of the tetrads, but more frequently to erroneous interpretation of the optical images produced by the homogeneous and more or less translucent chromosomes. I shall therefore first consider the various forms of chromosomes, the movements of their chromatids, their relation to the spindle fibers, and will then attempt an interpretation of the accounts given by other investigators.

The simple, unaltered, rod-shaped form is rarely seen in the metaphase for the reason that, as the chromosomes take their position in the equatorial plate, the points to which the fibers attach approach nearer the center, leaving the free ends to be drawn closer together at the periphery of the cell, as is shown typically in figures 2, 33, 46 and 92. While the statement is true regarding the infrequency of this form of chromosome viewed from the pole of the spindle in early metaphase, the opposite is the fact when chromosomes are regarded laterally in late metaphase or early anaphase. Under these conditions practically all the elements of the equatorial plate are long, rod-shaped chromosomes extended in the axis of the spindle. No matter what may have been the shapes of the chromosomes at the beginning of the metaphase they all are drawn out into rods just before they separate on their way to the opposite poles of the spindle. Lateral views of these conditions are represented in figures 8, 9, 10, 78, 96 and 97 where the complexes are extended in a line. That the same chromosome of two complexes may show itself lying in the equatorial plate in one and elongated at right angles to this position in the other may be seen by comparison of these groups.

While therefore the unmodified rod lying in the equatorial plate is rare, the form produced by bending in the plane of the longitudinal cleft to form V's and U's is very common. In *Mecostethus*, as may be seen by inspection of figures 32 to 34, 37, 41 to 44 and 46, practically all of the long, slender chromosomes are V- or U-shaped except such as extend across the plate, in which case they are more or less straight, simple rods. It is rare to find so much uniformity of shape as appears in this genus, but its significance with regard to the movements of the chromatids is great. Lateral views of the *Mecostethus* chromosomes at this time show that while the small ones are extended in a plane of the spindle axis (fig. 39) as in other species, the larger ones suffer an earlier separation of the chromatids and become diamond shaped with an open center (fig. 35) instead of linear and solid as in most cases. This is merely the precocious appearance of a chromatid separation which occurs in the early anaphase of all species.

Should the outer, divergent ends of the V-shaped chromosomes become approximated (or should they remain united, if the reverse is the character of the movement), annular chromosomes are produced. Much confusion regarding these structures exists needlessly in the literature. The method of their formation is clear and unmistakable and transitional forms are so common as to point definitely to the relations existing between them and the V- and U-shaped forms. In figure 2, for instance, the upper chromosome is a ring but the outer ends are only slightly joined. With just a little wider separation the form would be similar to the next chromosome to the left, a little more space and it would be shaped like the second one to the left, and with wider divergence of the ends would approach the type of the one below and to the right. In figure 2 there are at most two ringed-shaped chromosomes, while in figure 3, from the same animal, there appear six without divergent ends. It is evident that some of the chromosomes which in figure 2 are V's are, in figure 3, rings. Similar evidence is afforded by the chromosome groups of *Tropidolophus* shown in figures 21 to 30, in which the annular chromosomes vary in number from three to seven. As a rule the larger

elements tend to become ring-shaped, but sometimes fail to pass the V-condition and then may be identified with the same sized element in other cells. Such relations appear in the cells of *Tropidolophus*, just referred to, and also in cells from *Mestobregma* shown in figures 92 and 93.

In many cases the approximation of the outer ends of the chromosomes does not pass beyond their mere endwise fusion, but in some species there is a marked tendency for this portion of the chromosome to take on the same form as the inner end, or to exceed this and to form a second ring. In such cases the outer ring has its plane at right angles to that of the inner. Elements of this type appear in figures 21 to 26, 28 and 30. They are lacking in the cells represented in figures 27 and 29 from the same animal. A lateral view of an incomplete second ring is shown in the chromosomes at figure 25 b and at the right of the row in figure 97.

All the possibilities of form, due to movements of the chromatids within the plane of separation corresponding to the longitudinal cleft of the homologous chromosomes, have been considered, and mention has been made of the movement of these elements when they pass from the plane of the equatorial plate to that of the spindle axis. The latter condition is added to the other changes in form in varying degrees depending upon the extent to which it has already taken place in the prophase. During one or the other of these periods this gliding of daughter chromatids along the plane of their cleft and the subsequent approximation of homologous chromatids in a plane at right angles to this must occur. In *Mecostethus* all this transposition falls in the metaphase, for the elements start this period as rods or V's extended in the equatorial plate. Polar views of such stages are shown in figures 32 to 34, 37, 41 to 43, and 46, and in figures 35, 36, 39, 40 and 45 are lateral views. Figure 38 shows individual chromosomes from different angles with a prophase chromosome at the bottom for comparison. In this case it is demonstrated beyond any question that the separation of the elements is along the length of the chromosomes and that they recombine, for a time, by the approximation of homologous chromatids in the

plane of the spindle axis. The extremes of such a process are shown by two chromosomes lying side by side in one cell as represented in figure 64 e.

While for the sake of convenience in analysis the conditions of the prophase have been considered separately from those of the metaphase, the facts indicate that the changes in form are continuous and may proceed in varying degrees in different chromosomes during any one phase. For the same reasons the different chromatid movements have been reduced to types, but it is observed that they are rarely simple and that they occur in different combinations so that in most cases, in any one chromosome, it is necessary to resolve the changes into their components. When this is done for the metaphase chromosomes it is found that in general there is more or less bending or flexure in the plane of the longitudinal division combined with varying degrees of extension of homologous chromatids side to side at right angles to this. These two movements are sufficient to account for by far the greater number of metaphase chromosome forms and exclude the possibility of the occurrence of fundamentally different chromatid arrangements.

c. Relations of chromosomes to archoplasmic fibers

(1) As a part of the relations in the metaphase there must be considered those between the chromosomes and the archoplasmic fibers. It is not pertinent, in Orthopteran cells, to speak of the fibers attaching to the chromosomes as 'mantle fibers' for the reason that the spindle encloses the area occupied by the chromosomes and of itself constitutes a mantle (fig. 129). Nevertheless there are definite fibers attaching to the chromosomes and the relation thus established is most precise and constant. With but few exceptions fiber attachment is at the center of the rod or V-shaped chromosomes or, in the various modifications of this simple form, at the corresponding place, which is the point of fusion between homologous spermatogonial chromosomes. Directly related to the form of the tetrad and to the character of its movements is this fiber attachment, for it fixes the position of the chromosome

in the equatorial plate and guides the passage of the chromatids in their divergence. The result is that all of the chromosomes take up such a position that the synaptic points lie toward, or near, the spindle axis, and all of the chromatids execute like convergent movements toward their respective spindle poles. The position that the chromosomes take in the first spermatocyte metaphase and the position of their fiber attachment are seen, by a comparison with an equatorial plate of the spermatogonium, to be the same in both generations, the sole difference being that in the earlier generation each chromosome is free and independent while in the later they are joined by their corresponding inner ends into pairs. These conditions are clearly evident from a consideration of the two stages in a form like *Mecostethus*, as may be seen by comparing figure 44, representing a polar view of a spermatogonial complex, and figure 43 showing a like aspect of a first spermatocyte group. There are the same twenty-three elements in the same relative positions to the spindle and fibers, the one difference being the pairwise union.

From all these facts it is reasonable and fair to conclude that there is a typical organization of the tetrad which extends even to the relation which it bears to the archoplasm. In *Hippiscus* and most other Acridians this is of the character just described, but there are a few exceptions and these are of particular interest because of the strong evidence they give regarding precision of chromosome organization.

(2) In *Stenobothrus*, *Chorthippus*, *Chloëaltis* and *Trimerotropis* are found conditions that differ somewhat from the account which I have just given as characteristic of the Acrididae. These differences, at first suggestive of underlying, essential changes in type, are found on careful study not to be such, but only individual modifications of certain features by a few chromosomes. The same precision of organization obtains, but details are modified. Apparently the differences are due to changes in the position of the fiber attachments, but whether this is the result of inner structural modification of the chromosomes themselves or whether it is an archoplasmic change does not appear with certainty. Considering all the intrinsic permutations of the chromosomes, it

would seem probable that this changed external relation is the result of internal organization. Whatever be the cause the results are definite and exact.

During the prophase, indications of change are wanting until in the late stages, but upon the establishment of the full mitotic figure marked differences in appearance of certain chromosomes are at once apparent. These are perhaps not so prominent in polar views, although the familiar clear rings are wanting and the common V-shaped elements are few, but regarded laterally most striking differences at once appear. The prophase rings, which later were missing in their customary position in the equatorial plate when viewed from the pole, are very evident now, extended instead, at right angles to this position (figs. 63 and 64 a, 66), and there are unfamiliar E-shaped chromosomes (fig. 65) and asymmetrical rings (fig. 64 g). Occasionally there are rings lying in the plane of the equatorial plate where they form part of an E-shaped element, as is represented by the large chromosomes at the left of figure 63. There are also elongated rods with bent ends (fig. 64 b) and among these unusual forms appear the common rods and crosses (figs. 63, 64 e, 64 f, 65). The accessory chromosome, instead of being rod-shaped and going thus to the pole of the spindle, is V-shaped with fiber attachment at the center (fig. 66).

An analysis of these conditions, in comparison with the spermatogonial chromosomes which enter into the first spermatocyte, and with the second spermatocyte chromosomes which emerge from it, gives definite evidence of the cause underlying the changed conditions in these species. Primarily they seem to be due to a shifting of the fiber attachment from the end of the chromosome to the middle or to some intermediate point, as was described for the spermatogonia. Whatever the position of the fiber insertion in the earlier generation it is carried over into the first spermatocyte and appears again in the second spermatocyte. If this be at the center of the chromosomes when they join in synapsis, fusion takes place at both ends producing a ring with a possible divergence of the chromatids at each contact end. If such lateral extensions occur they must disappear later when the halves

of the chromosomes separate. The two conditions are shown in figure 64 a, being a drawing of chromosomes from the same cell, and in figure 66. Occasionally rings are formed when the fiber insertion is not median, and in that event divarication of the chromatids takes place only on the side of the larger members (fig. 65, next the E-chromosome) or it may be that the opposite result is reached (fig. 64 g). More commonly however, when the fiber attachment is subterminal, rings are not formed and synapsis occurs between the longer limbs only. This is commonly true of the larger pair of spermatogonial chromosomes and the result is the E-shaped chromosome or some modification of it. In this element it not infrequently happens that the divergent ends, at the point of fusion, become much extended in the equatorial plate at the expense of the arms attached to the fibers, and swing round in the form of a ring. This part when viewed laterally appears as a much extended middle bar of the E, but when seen from above exhibits the ring form modified in appearance by the bent arms above and below.

All the evidence indicates that the composition of the tetrads is the same in all of the Acrididae studied. There are the same elements of the tetrads to be distributed in each case. It is therefore apparent that the method of this distribution must differ in *Stenobothrus* and other similar forms, with rings extended in the axis of the spindle, from that in such as have rings lying in the equatorial plate. A comparison of the annular chromosomes of these two types is most suggestive and seems to offer unquestionable evidence of the distinction between chromosomes that suffer a reduction division in the first spermatocyte and those that are divided equationally. In considering the work of different investigators upon the question it will be necessary to enter into this comparison more fully, but in order to give a basis for this I will here briefly note what appear to be essential elements involved in the discussion.

(1) The composition of the rings is the same in all cases and the point of fiber attachment is fixed for each chromosome.

(2) Rings that divide longitudinally in the first spermatocyte lie extended in the equatorial plate and have the fiber attachment

at the synaptic end, which lies toward the spindle axis. In the anaphase the daughter chromosomes go to the pole as simple V's (figs. 5, 6, 7 and 35).

(3) Annular chromosomes dividing transversely lie extended parallel to the spindle axis and have the fiber attachment at the middle of the chromosome or near the middle. The anaphase chromosomes are double V's (fig. 67).

It is necessary also to consider those other chromosomes with median or subterminal attachment of fibers which do not take on the form of rings. For our present purpose it is sufficient to note that they differ only in the degree of their chromatid movements from the rings and in the late prophase are indistinguishable from them. The chromosomes shown in figures 64 b, c, d and h may have been rings like figure 64 g or their parts may never have come into the ring association. The points of interest to note here is that such chromosomes, whether rings or not, always show in the anaphase double V-shaped forms.

CHROMOSOMES OF SECOND SPERMATOCYTE METAPHASE

The conditions of most interest for this study with regard to the second spermatocyte reveal themselves fully only in the metaphase condition, but it is necessary to note briefly some of the earlier phases. Of course, so far as the accessory chromosome is concerned, it is found in but half this generation of cells, but, since this does not otherwise influence the chromosome relations, it will not be necessary to consider the matter further at this time. The distinction between the first spermatocyte telophase and the second spermatocyte prophase cannot be sharply drawn, for the reason that in some species there is no loss of form by the chromosomes, and the only indication of the prophase condition is the presence of the chromosomal group within a nuclear membrane and the forming archoplasmic spindle. This period is very brief and it is possible to find exceptionally in one cyst first spermatocyte telophases and second spermatocyte metaphases.

A second spermatocyte metaphase figure distinguishes itself at once from that of either of the two preceding cell generations,

and it is most unlike that of the first spermatocyte. In the size and form of the spindle and shape of the chromosomes it most resembles the spermatogonial mitotic figure, but differs in the possession of the haploid instead of diploid number of chromosomes. While it is like the first spermatocyte in number of chromosomes it is most unlike in the form of the chromosomes and spindle. When viewed from the pole the second spermatocyte metaphase figure shows all the elements of like form—simple rods with terminal fiber attachment and arranged radially in the equatorial plate (figs. 12, 13, 16 to 19, 31, 47, 49, 50, 53 to 58). A lateral view reveals each chromosome as two rods superimposed with the inner ends, where the fibers attach, in close contact, while the outer ends may be widely divergent (figs. 48, 51 and 52). Occasionally chromosomes lying in the center of the group may swing widely apart, remaining united only at the point of fiber attachment and may then appear almost as straight rods. Such instances are represented in figures 18, 49, 50, 53, 55, 57 and 95.

In the cases of *Stenobothrus*, *Chorthippus*, *Chloëaltis* and *Trimerotropis*, where some of the chromosomes have median and subterminal fiber attachment in the spermatogonia, a similar series of such chromosomes reappears in the second spermatocytes. Under these conditions polar views of the second spermatocyte metaphase (figs. 68 to 76) instead of presenting all the chromosomes as simple rods will show V's with equal or with unequal arms, depending upon the point of fiber attachment. Divergence of the outer ends also occurs to some extent in such chromosomes.

DISCUSSION OF RESULTS ON THE SPERMATOCYTES

In weighing the evidence presented in the different accounts of Orthopteran maturation phenomena it will be necessary to take into consideration the following items; (1) the relation of the chromosomes of the first spermatocyte to those of the spermatogonium, (2) the composition and form of the first spermatocyte chromosomes, (3) the relation of the chromosomes of the first spermatocyte metaphase to those of the prophase, (4) the position of the chromosomes in the spindle, noting the *Hippiscus* type and

the *Stenobothrus* type of chromosomes, (5) the movement of the chromatids in the metaphase in these two types, (6) the relation of the chromosomes to the archoplasmic fibers in the two types, (7) the form of the anaphase chromosomes in *Hippiscus* and *Stenobothrus* types, (8) the results of the first spermatocyte mitosis under the conditions of the two types, in terms of the chromosomes, (9) the results of the second spermatocyte divisions in the two types.

Because of the involved and contradictory nature of the various accounts it has seemed best to review briefly the work of the different authors, individually, according to the analysis proposed, and then to consider the general evidence on each of the points. By this means it will be possible to exclude the palpably incorrect portions of the various descriptions and to present for judgment those possibly correct ones upon which there is difference of opinion. The papers on each family will be grouped together.

a. Acrididae

Wilcox ('95) in his paper upon the spermatogenesis of *Melanoplus* (*Caloptenus*) *femur-rubrum* describes the behavior of the chromosomes in such a way that it is quite impossible to compare his account in detail with more exact studies. The attempt to do this will nevertheless indicate somewhat the advance that has been made since his work was done. (1) The 12 univalent chromosomes of the spermatogonium become extended into a granular thread upon which the chromatin collects at twenty-four points, and then the thread breaks transversely into 12 dumb-bells. (2) By joining these dumb-bells in pairs, 6 tetrads are produced, making 24 chromosomes. These are all in the form of rings. (3) Contraction results in the production of metaphase chromosomes having also an annular shape. (4) The rings are placed on the spindle parallel to its axis. (5) By the first spermatocyte mitosis they are separated into half rings. (6) No clear statement of fiber attachment is given. (7) Chromosomes in the anaphase are not described. (8) The first spermatocyte mitosis is a reduction division. (9) Also the second spermatocyte division is reductional.

The work of de Sinéty ('01) upon *Oedipoda*, *Stenobothrus*, and certain *Phasmids* was not done with a detailed consideration of chromosome relationships and so it is not possible to bring it strictly into line with more recent studies. Such a comparison will however be valuable in showing the greater precision of our present knowledge: (1) No adequate attempt is made to analyse the members of the first spermatocyte complex in terms of the spermatogonial chromosomes. (2) The first spermatocyte chromosomes are formed by the transverse division of the chromatin thread, which then divides twice longitudinally in planes perpendicular to each other. There result, by different degrees of separation and movement of these four parts, chromosomes in the shapes of rings, loops, crosses, etc. (3) Through concentration these become the final metaphase chromosomes in which the second longitudinal split is lost. (4) Because no distinction is made between the *Stenobothrus* and *Hippiscus* types it is not possible to compare the behavior of the various chromosomes in the spindle. (5) No uniformity in the movements of the chromatids in metaphase can be deduced from de Sinéty's account except that they separate along the first longitudinal cleft. (6) The fiber attachment may be terminal, median, or subterminal. Differentiation between conditions in *Stenobothrus* and *Oedipoda* is not made. (7) Forms of chromosomes in the first spermatocyte anaphase depend upon the position of fiber attachment and may be V-shaped with terminal attachment, double V-shaped with median and double J-shaped with subterminal. (8) The first spermatocyte division is longitudinal in the plane of the first split. (9) Along the second longitudinal cleft the chromosomes separate in the second spermatocyte. There are accordingly two longitudinal divisions and no reduction.

Montgomery ('05) confined his Orthopteran studies to a single species, *Syrbula acuticornis*, and came to a number of conclusions very different from those reached by other investigators, although in general the processes were conceived to be similar to those in other *Acrididae*: (1) Twenty chromosomes were reported for the spermatogonium, of which one pair constitutes the heterochromosomes. These pairs might be distinguished by differences in

size and form. By end to end synapsis these 20 chromosomes become reduced to 10, among which the heterochromosomes are indistinguishable from the tetrads. There is a persistent chromosome individuality manifested. (2) The various forms of prophase chromosomes reported by other cytologists appear, according to Montgomery, but X's are rare. Rings are not uncommon at this stage. (3) Forms of chromosomes recognizable in the prophase may appear in the metaphase of the first spermatocyte, but such forms as rings, common in the prophase, are rare in the metaphase. Whatever the form, however, a common type is recognizable, i.e., two spermatogonial chromosomes united end to end and having fibers attached to the opposite ends. (4) In the mid period of mitosis the chromosomes take up such a position that the fused ends lie in the equatorial plate. Rings are found only exceptionally, as are X's. (5) The first maturation division sees only a separation of whole, univalent spermatogonial chromosomes. Such as lie extended in the equatorial plate are pulled past each other to an opposite pole of the spindle. (6) Fiber attachment is at the ends of the chromosomes opposite the synaptic fusion. (7) In the anaphase the chromosomes all have the form of V's in which the enclosed space represents the earlier longitudinal split. (8) A reduction division of all the chromosomes is accomplished in the first spermatocyte where the heterochromosomes behave as do the other tetrads. (9) Also in the second spermatocyte all the chromosomes divide in the same way, so that a mere equational division results. This includes the heterochromosomes.

Davis ('08) has made a careful and extended study of a number of Acrididae and Locustidae and finds a common type of maturation processes in all. For the Acrididae he chooses, as representative, the conditions found in *Dissosteira*, and includes also a consideration of *Stenobothrus*. For the Locustidae *Steiroxys* is employed. From this diverse material he concludes that (1) the spermatogonial chromosomes may be recognized in pairs, with the exception of the accessory chromosome, and these reappear in the first spermatocyte, joined end to end, to form the tetrads. (2) Chromosomes of the first spermatocyte are all of the

same type of construction, but show a variety of shapes which are constant in number. Thus in the Acrididae there are always three rods, two very small and one medium in size; three crosses, two of medium size and one large; and five rings or loops, four of which are large and one of medium size. In *Stenobothrus* similar constant shapes, but in different numbers, occur. In every case the chromosome is formed by the end union of spermatogonial chromosomes and, if unmodified, produces the simplest type—the rod. If, at the point of union in such an element, the parts diverge and become extended at right angles to the length of the rod, the cross is formed. Rings represent a union of the two spermatogonial elements at both ends instead of at one as in the other types. When the fiber attachment is not terminal, as in *Stenobothrus*, there may be a lateral extension of the parts, as in the rods, joined to a flexure at the point of fiber attachment forming E-shaped chromosomes. (3) A gradual condensation of the chromosomes carries the forms of the prophase over into the metaphase. (4) In the first spermatocyte spindle the rods are placed parallel to its length, the crosses with one arm in the direction of the spindle axis and the other in the equatorial plate, while the rings and loops are in such a position that their free ends lie central and crossed with the synaptic ends directed peripherally. According to the description homologous chromosomes should lie one above the other so that the opening of the ring would be apparent from a lateral view, but in the few metaphase figures shown (text fig. N) they appear clearly in polar views. The figures of *Stenobothrus*, upon which form Davis clearly has established his views, are much more numerous at this stage and the rings are accurately shown lying extended in the direction of the spindle axis. The other chromosomes appear as in *Dissosteira*. (5) At the time of chromatid separation the halves of the extended rods merely move apart, showing first a constriction at the point of division; the crosses suffer a reduction of the lateral arms, due to an extension of their substance into the vertical arms, and at length come to have the same shape as the rods. The rings have their curved halves pulled past each other in the axis of the spindle and at the end of the

movement are extended in this direction as split rods. Where the fiber insertion is not terminal, as in *Stenobothrus*, the rings separate as longitudinally divided half rings. (6) Davis conceives the point of fiber attachment to be permanent from generation to generation of cells, and finds strong confirmation for this idea in the chromosomes of *Stenobothrus* where there are terminal, median and submedian attachments. In *Steiroxys* there are also median attachments, but in the other forms studied apparently they are all terminal. (7) Where the fiber attachment is terminal the anaphase chromosomes of the first spermatocyte appear as simple V's, but in those cases with median or subterminal attachment the chromosomes are double V's or J's. (8) As a result of his study on the different species of Orthoptera, Davis concludes that the first spermatocyte mitosis witnesses a separation of homologous chromosomes and is therefore a reduction division in Weismann's sense. (9) Further he concludes that in the second spermatocyte the halves of chromosomes, longitudinally split in the first spermatocyte prophase, are separated by an equatorial division.

Buchner ('09), from his investigation of Oedipoda, reaches the following conclusions: (1) The twenty-three chromosomes of the spermatogonium are recognizable as eleven tetrads, plus the accessory chromosome, in the first spermatocyte. (2) The planes of the future longitudinal and cross divisions are recognizable in the prophase chromosome, which is an elongated thread with a central cleft representing the position of the future reduction division. This extended thread may be subjected to unequal pressure on the two sides and then takes the form of a U. The free ends of such a tetrad may come into contact and thus produce a ring. In oblique view these rings, which do not lie in one plane, have the appearance of a figure 8. By approximation of the two elements lengthwise and by breaking apart at the ends, parallel rods result. If the forces exert themselves equally on both sides of the tetrad it remains extended. Short elements under these conditions are straight rods, but longer ones may be drawn out at the center and produce crosses with arms of various proportionate lengths. Combinations of the two types may occur and

rings produced by the first form of movement may have the elements drawn out at the center producing a cross on a ring. Such secondary crosses do not, however, occur on both sides of the ring. (3) By continued contraction the various forms become more uniform and with the loss of the nuclear membrane there may be found round and elongate bodies corresponding to the earlier rings and crosses without any indication of central opening or longitudinal split except in the largest rings. (4) Upon the establishment of the spindle there are found, in lateral view, chromosomes with their longitudinal axis parallel to the spindle and others at right angles to it, with all intermediate forms. The conclusion is that "die Stellung der extremen Chromosomen gestattet uns den Schluss, das die Teilungsebene übereinstimmt mit der Ebene, in der die Chromosomengrenzen liegen; die erste Reifeteilung stellt eine echte Reduktionsteilung (Praereduktion Korschelt-Heider) dar." (5) Buchner fails to observe the movements of the chromatids while in the equatorial plate. (6) One fiber attaches to each chromosome at a clearly marked point, and this varies in position in each chromosome. (7) The form of the anaphase chromosomes is variable depending upon the position of the fiber attachment. No indication of the longitudinal cleft—or plane of second spermatocyte division—is visible, but it runs the length of the arms of the anaphase U's. (8) All chromosomes are divided transversely in the first spermatocyte and the various forms of anaphase chromosomes go over into the second spermatocyte with an invisible longitudinal cleft. (9) After a brief resting period and prophase, in which the anaphase chromosomes may be recognized, the second spermatocyte metaphase figure is formed with the chromosomes, longitudinally divided, lying in the equatorial plate. They are all of one type with terminal fiber attachments and divide along the plane established by the longitudinal division of the first spermatocyte spireme. The anaphase shows no V-shaped chromosomes.

Brunelli ('09, '10, '11) has studied the maturation phenomena in *Gryllus desertus* Pall. and in *Tryxalis* and concludes that, in essentials, they are similar. For *Tryxalis* ('10, '11) he states

that (1) it is difficult to determine exactly the spermatogonial number, but it is probably 21 although his figure 17 shows 23 very clearly. In the first spermatocyte, however, there are 10 tetrads plus the accessory chromosome. There is an individuality of the chromosomes and spermatogonial elements reappear in the first spermatocyte joined in pairs. (2) There are various forms of the chromosomes due to the movements of their parts as well as to the different aspects from which they are viewed. They are conceived to be rods joined at one end and variously twisted and separated. The union is slight and often they lie across each other or one is bent around the other. Rings are loops, although in figure 11 a perfect annular element is shown, while crosses are formed of overlying parts. (3) The forms of the prophase are traced into the metaphase. (4) No clear polar views of the metaphase are given, but in later views the paired elements are shown placed one above the other—'superposition'—with their inner ends crossed. (5) In the dicussion of the chromosomes they move past each other going to the opposite pole of the spindle from that side of the equatorial plate upon which they lay in early metaphase. Whole spermatogonial chromosomes are thus separated from each other by simply being pulled apart. (6) Fiber attachment is terminal and to the spermatogonial derivative lying on the *opposite* side of the equatorial plate. (7) The anaphase chromosomes are single V's whose contained space is the longitudinal division established even as early as during the anaphase of the preceding spermatogonial division. (8) By the segregation of whole chromosomes in the first spermatocyte there results a prereduction type of maturation. (9) The V-shaped chromosomes of the anaphase are traced through the interkinesis and into the second spermatocyte metaphase where they are divided along the longitudinal cleavage of each, thus constituting an equation division.

Gerard ('09) has studied only *Stenobothrus* and his results may be compared in detail only with those of other investigators upon similar material. Summarized, his findings are that (1) while there is a loss of chromosome outlines after the last spermatogonial division and the formation of a network, there subse-

quently appear two spiremes which, by parasynapsis, become a double one, and this then divides by transverse fission into the reduced number of chromosomes, 17. These are of the sizes found in the spermatogonium and represent them in the paired condition. (2) The forms of chromosomes in the first spermatocyte prophase are rings, figures of 8, etc., in which the open space represents the plane of fusion between the spiremes—it is interchromosomal. (3) By concentration the metaphase chromosomes are formed from those of the prophase. (4) Upon the spindle the rings are so placed as to lie extended in its axis. Rods also occur in the same position. (5) The rings break into half rings or V's whose enclosed space is the interchromosomal cleft between the spiremes. (6) There is no detailed consideration of fiber attachment. (7) In the anaphase there appear double V's whose separation is due to the longitudinal split of the chromosomes. (8) Through the movements of the chromosomes in the first spermatocyte mitosis a reduction division is accomplished. The accessory chromosome does not divide at this time. (9) In the second spermatocyte an equational division occurs.

Granata ('10) has studied for the maturation phenomena of the chromosomes the material employed by Giglio-Tos in his investigations of mitochondria, which was derived from the European genus *Pamphagus*. As has already been noted in considering the spermatogonia, this form appears to show different numerical conditions from most other Acrididae. The conclusions of Granata are as follows: (1) The number of chromosomes is constant and the members appear as paired elements of definite sizes. An unpaired accessory chromosome is present. A spireme is formed in which the spermatogonial chromosomes lose themselves. There is no persistent chromosome individuality since these structures are but the expression of chemical activities. (2) The typical first spermatocyte chromosome is the cross formed from a rod which has been twice divided longitudinally and has then opened out part way along one of these clefts and later, from the opposite end of the rod, along the other. Rings are similar structures which, instead of opening out completely along the second longitudinal cleft, have remained united at the

end while diverging in the center. Figure-8 chromosomes are such rings with a secondary divarication of the end opposite the first producing a second ring at right angles to the primary ring. (3) The definitive metaphase chromosomes reach their forms by a condensation of similar figures in the prophase. (4) No clear statement regarding position of the chromosomes in spindle is given but figures show rings lying in the equatorial plate and the statement is made that the author agrees with the descriptions of McClung and his students. (5) A similar statement may be made regarding the movements of the chromatids in metaphase. (6) The relation of chromosomes to fibers is not clearly stated. (7) In the anaphase the chromosomes pass to the poles of the spindle as simple V's. (8) The result of the first spermatocyte mitosis is a longitudinal division of the chromosomes and no division of the accessory chromosome. (9) The telophase chromosomes may be traced into the second spermatocyte and later are divided along the second longitudinal cleft, while the accessory chromosome divides lengthwise also.

Meek's work ('11) deals with the case of *Stenobothrus* only. His conclusions are that (1) while there appears to be a complete loss of chromosome identity during the growth period, the same series of sizes and shapes reduced to the haploid number 9, reappears in the first spermatocyte metaphase and it is possible that the chromosomes of this stage are formed by earlier conjugation of homologous spermatogonial elements. (2) Each first spermatocyte prophase chromosome is formed of four parts, and may show a variety of shapes—crosses, rings, loops, figures of 8, etc. (3) The metaphase chromosomes appear either as rods, or as V's with more or less unequal arms. (4) On the first spermatocyte spindle the chromosomes are placed principally as extended rods or crosses. (5) No clear account is given of the movement of the chromatids in the metaphase. (6) There is no statement regarding fiber attachment. (7) An account of the anaphase chromosomes is not given. (8) The first spermatocyte division is thought to be longitudinal. (9) In the second spermatocyte a possible reduction division occurs, although it is conceived that both spermatocyte divisions may be longitudinal.

b. Locustidae

Stevens ('05), in connection with a series of studies upon the accessory chromosome, describes briefly the spermatogenesis of *Stenopelmatus*, a Locustid. Several errors appeared in this paper which were later corrected. Such of her final conclusions as relate to this study are indicated in the following: (1) From the 47 chromosomes of the spermatogonium there are formed by telosynapsis in the prophase of the first spermatocyte twenty-three tetrads and an unpaired accessory chromosome. (2) The tetrads thus produced have the form of rings, crosses and rods. (3) In the metaphase the same series of forms may be traced. (4) The chromosomes are so placed in the spindle that their plane of longitudinal cleavage lies in the equatorial plate. This is clearly indicated by persisting linin connections. (5) By movement of the chromatids along the plane of the longitudinal split in the late metaphase, the chromosomes have the form of rods extended in the direction of the spindle axis. (6) Fiber attachment is not described, but by inference it must be at the synaptic points. (7) Anaphase chromosomes are in the form of simple V's or double rods. (8) An equational division occurs in the first spermatocyte. (9) Segregation of homologous chromosomes results from the second spermatocyte mitosis.

Otte ('07) has confined himself to a study of the Locustid, *Locusta viridissima*, and comes to some unique conclusions regarding maturation phenomena. His results may be summarized as follows: (1) There is a constant series of chromosomes recognizable by differences of size. The paired elements of the spermatogonium, after becoming elongated in the first spermatocyte prophase, join together side by side to form the haploid number—parasynapsis. (2) Of these, the longer elements become rings by a bending of the double thread until the ends meet, at which point they may, or may not, fuse. These annular chromosomes may continue in this form or they may become compressed so that they break at the point of greatest curvature—opposite the free ends of the loop—and thus produce parallel rods, each of which shows a space representing that produced by the earlier ap-

proximation of the two threads. Smaller elements may fail, or be unable, to form rings and then appear as straight rods. Such rods then break across at the middle and form tetrad-like chromosomes. Sometimes at the middle point the halves of the threads are drawn out to form cruciform chromosomes. (3) The various prophase forms are traced directly into the metaphase through a gradual condensation. (4) The rings lie in the spindle parallel with its axis; the rod-shaped forms are extended in the same manner. (5) A mere separation of the half rings or half rods is all that occurs in the metaphase. (6) In the case of the rings, fiber attachment is at the middle of each half ring, in the rods at opposite ends of the extended structure. (7) Forms shown by the anaphase chromosomes are split half rings from the annular chromosomes and V's from the rods. (8) By the first spermatocyte division one end of each of the spermatogonial chromosomes, joined side by side in the prophase, is separated from the other end and goes into a different second spermatocyte. It is not a reduction or segregation division because homologous elements are not separated; it is not an equational division for the reason that not all parts of each chromosome are represented in the daughter cells. As described by Otte it is an entirely unique form of division. (9) The second division is like the first, even to the forms of the chromosomes. Before the second spermatocyte metaphase the half rings have again become rings and are once more separated as in the preceding mitosis. The rods are again divided transversely and go to the poles as split rods. This also is neither a reduction nor an equational division. The result of the two divisions is to divide each spermatogonial chromosome, joined by parasynapsis to its homologue, twice transversely, one-fourth of its length going into each spermatid.

Stevens ('12) discusses supernumerary chromosomes and the method of synapsis in *Ceuthophilus*. She finds a parasynapsis, subterminal fiber attachment and a reduction division in the first spermatocyte.

Vejdovský ('13), together with studies upon *Ascaris*, *Gordius* and other genera, has reported the results of his investigations upon *Decticus* and *Diestramena*, two Locustid genera. His

results may be stated as follows: (1) There is constancy in number of chromosomes with differential sizes in a duplex series. After the last spermatogonial division the chromosomes lose their linin center and the naked chromatin threads of homologous chromosomes fuse their substance to produce a new mixochromosome. (2) These mixochromosomes early split longitudinally to produce dyads. Just before the first spermatocyte metaphase these dyads again divide longitudinally to produce tetrads, the largest of which are rings or ellipses. (3) These tetrads, by slightly more concentration, pass into the metaphase where the rings are large and conspicuous. (4) These rings are of the Stenobothrus type and lie parallel to the spindle axis. (5) In the metaphase the annular chromosomes are reduced to half rings by dividing across at their middle. (6) The fibers attach at the centers of the half rings. (7) The anaphase chromosomes resulting are double half rings or V's. (8) As a result, the first spermatocyte mitosis represents a longitudinal division. (9) In the second spermatocyte the chromatids are separated along the second longitudinal cleft. There are, therefore, two longitudinal divisions and no reduction.

c. Gryllidae

Vom Rath ('91, '92, '95) has reported the process of maturation in *Gryllotalpa vulgaris*, but, as stated in the discussion of the spermatogonia, his results are so evidently erroneous that it is profitless to attempt a comparison between them and the findings of others. It is only necessary to mention, to indicate the character of his work, that he reported the diploid number of chromosomes to be 12 instead of 23, and that he entirely overlooked the prominent accessory chromosome. For these reasons his work will not be considered further.

Guthertz ('07, '08, '09) in a series of papers on *Gryllus* has discussed the behavior of the accessory chromosome and its relation to sex determination, but his work does not concern itself closely with the problems of this paper. He notes the typical behavior of the accessory chromosome during spermatogenesis

and denies the presence of such an unpaired element in the female cells.

Voinov ('04) deals only with the behavior of nucleolar bodies.

Brunelli ('09) states in this paper many of the views which he elaborated later in his study of *Tryxalis*. Briefly, he finds the 21 spermatogonial chromosomes of *Gryllus* converted into 10 tetrads plus the accessory chromosome. The prophase chromosomes may be interpreted as double rings, which in the metaphase, take on a variety of forms due to the point of fiber attachment and to the fluidity of the chromatin. The form in the metaphase may vary from that of the prophase for these reasons. In the metaphase the chromosomes are arranged in 'superposition,' and the half rings, representing spermatogonial chromosomes, separate. Fibers attach at the center of the half rings and the anaphase chromosomes are split V's. The first spermatocyte mitosis is reductional and the second is equational.

Payne ('13) in a short paper gives an account of the behavior of the accessory chromosome in *Gryllotalpa vulgaris* and describes an unequal tetrad, the large member of which always accompanies the accessory chromosome in its unipolar movement in the first spermatocyte mitosis. For our present purpose we may note that the diploid number, 23, is reduced to the haploid, 12, in the usual manner so as to leave an unpaired accessory chromosome. Baumgartner ('11), in an abstract of a paper read before the American Society of Zoologists, reports similar conditions in *Gryllotalpa borealis*.

d. Blattidae

Farmer and Moore ('05), after a study of a number of plants and animals, conclude that there is a typical series of processes, common to all multicellular organisms, in the reproductive cycle. Among the forms studied was *Periplaneta americana* and the processes of maturation in the male are thus conceived: (1) The chromosomes are morphologically continuous from generation to generation and the reduced number of the first spermatocyte is derived from the diploid spermatogonial series by fusion of homologous pairs. Details of this process are not clearly given

but it would appear from several statements that this must be due to union of chromosomes, and not to a unique cross-splitting of a continuous spireme. On page 527 this statement occurs: "It does not seem to be a matter of any consequence how the bivalent arrangement is produced, since there is so much variability in the process, but the temporary union in pairs of somatic chromosomes is the really important feature." (2) For *Periplaneta* the analysis of the tetrads into their univalent parts is not made clearly, but the idea of variability of association and dissociation is held. How much this means for these authors may be gathered from the description of the different methods by which the common X-shaped chromosome is produced in *Os-munda* (p. 522). At least three variations in constitution are mentioned. In *Periplaneta* the prophase chromosomes are in the form of loops with their free ends polarized at one side of the nucleus. A chromatin nucleolus is present. (3) The metaphase chromosomes are in the form of rings and are produced by approximation of the free ends of the loops. (4) Upon the spindle the rings are so arranged that they lie in its axis. They are therefore of the *Stenobothrus* type. (5) In the metaphase the annular chromosomes are separated into half rings. (6) Fiber attachment is median as in the spermatogonia. (7) The anaphase chromosomes are irregular half rings, or V's, with the original longitudinal cleft of the chromatin thread dividing them into double elements. (8) As a result of the first spermatocyte division the reduction, or segregation, of the biparental chromosome groups is effected. (9) After a very nearly complete rest stage the reduced number of chromosomes reappears in the second spermatocyte metaphase and there they divide "in the ordinary premaiotic manner."

Stevens ('05) describes the maturation phenomena of (*Blattella*) *Blatta germanica* with these results: (1) The 23 chromosomes of the spermatogonium become converted by telosynapsis into 11 tetrads, leaving the unpaired accessory chromosome. (2) Rings, crosses, U's and rods occur in the first spermatocyte prophase but they are of essentially the same type of construction. (3) Variation disappears in the metaphase and all the

tetrads are dumb-bells; (fig. 125 seems, however, to show rings very clearly). (4) All chromosomes lie with their longitudinal axes parallel to that of the spindle. (5) A mere separation of the dumb-bells marks the changes in the metaphase. (6) Fiber attachment is not discussed, but from the figures it appears to be terminal. (7) The forms of the anaphase chromosomes are not described or clearly figured. (8) In the first spermatocyte a segregation, or reduction, division occurs. (9) After a complete rest stage the chromosomes of the second spermatocyte appear and divide, presumably equationally.

Wassilieff ('07) says that in *Blatta germanica* the unreduced number of chromosomes is 23, of which one is the unpaired accessory chromosome. By some means, not fully explained, the haploid group of 12 chromosomes is produced from the chromatin of the nucleus resulting from the last spermatogonial division; (in explanation of fig. 37, rods are described as being chromatin segments conjugated end to end). One of the twelve behaves in many ways like a nucleolus and later passes undivided to one pole of the first spermatocyte spindle. The first spermatocyte prophase chromosomes have the form of rings or V's which are produced by the bending of a longitudinally split, transversely cleft thread upon itself. The two segments are separated at this cross division in the first spermatocyte, which is therefore a reduction division. Along the longitudinal split occurs an equation division in the second spermatocyte.

Morse ('09) records the consistent phenomena apparent in four genera of Blattidae. Among his observations the following of interest in the present study may be noted: (1) The chromosomes of the first spermatocyte are derived from the spermatogonial series by a parasynapsis of homologous members, leaving an unpaired accessory chromosome. (2) By an enlargement of the interchromosomal space rings are produced, but persistent rods also occur. (3) Condensation and contraction of these result later in the production of similar forms in the metaphase. (4) In the spindle the rings are arranged parallel with its axis; rods lie in the equatorial plate. (5) Annular chromosomes divide directly into half rings or V's after more or less approximation of the two

extremities in the equatorial plate; rods are pulled apart by gliding along each other in the equatorial plate until separated. (6) Fiber attachment is either median or subterminal. (7) Anaphase chromosomes are U's or V's for median fiber attachment, or J's in subterminal. (8) The first spermatocyte division is reductional. (9) In the second spermatocyte an equational division occurs.

e. Phasmidae

Jordan ('08 a, '08 b) presents a study of the spermatogenesis of the Phasmid, *Aplopus mayeri*, in which he deals at length only with the accessory chromosome. This is typically Orthopteran in its behavior. Additional facts having relation to maturation processes are that the 35 chromosomes of the spermatogonium become reduced during synapsis to 18, of which one is the accessory chromosome, and that these undergo a prereduction.

As a part of a comprehensive study of Phasmid structure de Sinéty ('01) reports the maturation phenomena in the male of several species. Since his conclusions have been discussed in connection with Oedipoda they will not be taken up here.

f. Forficulidae

Zweiger ('07) reaches the following conclusion from his study of the spermatogenesis of *Forficula auricularia*: (1) The haploid number of chromosomes, 12 or 13, is reached through a telosynapsis of the 24 or 26 chromosomes of the spermatogonium. (2) Tetrads in the form of rings are produced by the folding of longitudinally split bivalents joined end to end. The enclosed space is interchromosomal. (3) Concentration of the prophase rings results in the formation of dumb-bell shaped metaphase chromosomes. (4) These are placed on the spindle so that homologous chromosomes lie superimposed. (5) In the metaphase these are merely separated from each other. (6) Position of fiber attachment is not described but it must be median. (7) The anaphase chromosomes are spherical bodies. (8) By separation of whole spermatogonial chromosomes the first spermatocyte mitosis becomes a reduction division. (9) In the second

spermatocyte homologous chromosomes divide longitudinally and so undergo an equational division.

Randolph ('08) gives an outline account of the spermatogenesis in the earwig, *Anisolabis maritima*, dealing principally with the accessory chromosomes which are regarded as similar to an equal pair of idiochromosomes. No detailed description of chromosome behavior is given except that the 24 spermatogonial chromosomes become reduced to 12.

Stevens ('10) gives the results of her studies upon the spermatogenesis of *Forficula auricularia*. Briefly stated, her conclusions are that the diploid chromosome number, including an unequal idiochromosome pair, is 24. This, by a telosynapsis of the typical insect character, produces a haploid number of 12. The first spermatocyte prophase shows the chromosomes in the form of loops, U's, rings and figures of 8, with an occasional cross. A reduction division follows after the concentration of the chromosomes. Occasional variations in the number of second spermatocyte chromosomes are ascribed to precocious division of the small idiochromosome.

Sutton ('00, '02, '03) Baumgartner ('02), Nowlin ('08), Pinney ('08), Robertson ('08) and Carothers ('13) have studied various species of Orthoptera in my laboratory and their interpretations do not differ from mine in any essential respects. For this reason I shall not enter further into a consideration of their work here.

CONCLUSIONS

With the results of the various investigators before us it is now possible to draw comparisons and to arrive at conclusions. Such comparisons may advantageously be made under the various headings that have been used in outlining the results of individual investigations.

a. Relation between chromosomes of the first spermatocyte prophase and those of the spermatogonium

1. Concerning the relation existing between the chromosomes of the first spermatocyte and of the spermatogonium there are three theoretical possibilities. (a) The chromosomes of the

first spermatocyte may be entirely new structures, having no relation either in material or in form to those of the spermatogonium, (b) they may be derived in substance only from the spermatogonial elements, or (c) they may individually be descended from similar structures without loss of identity other than that involved in the processes of chromatin metabolism. All these conceptions have found expression in studies on Orthopteran material.

Granata ('10), adopting the views of Giglio-Tos, argues for a purely chemical explanation of chromosome behavior and so removes his interpretations from a direct comparison with the work of others. Despite this extreme view he is constrained to admit the presence of a chromosome series, constant in number and in size relations, with an exact reduction of the euchromosomes² to the haploid number of maturation. No other investigator has made an appeal from morphological conceptions and since Granata's interpretation is purely theoretical it need not be discussed further, while so much remains to be done by observation.

For the second possibility there are more advocates, although it is often rather by inference than by exact statement that this appears in the accounts. For while some profess a belief in the genetic continuity of the chromosomes they yet describe a double longitudinal division or a double cross division. Persistent chromosome individuality is, however, possible only in the presence of a segregation, or reduction division during maturation. With this understanding of the case Wilcox and Otte, conceiv-

² The word 'euchromosome' I shall employ to distinguish those without any marked peculiarities of form or behavior. Montgomery's term 'heterochromosome' might advantageously be used as an antonym, signifying any general variation of structure or behavior. Subclasses within the general term heterochromosomes could be constituted under the headings 'idiochromosomes' (Wilson) to include paired sex chromosomes; 'accessory chromosome' (McClung) to distinguish the unpaired sex element; 'microchromosomes' (Wilson) to designate small compact chromosomes not connected with the sex chromosomes. I believe it much preferable to use the genetic word 'chromosomes' in all terms denoting such cell elements and to mark their specific differences by qualifying terms, rather than to coin entirely new names for them in which no intimation of their relations is included.

ing the presence of a double cross division, and de Sinéty and Vejdovský a double longitudinal division, must be recorded as upholders of a mere substance relation between the chromosomes of the two generations of germ cells. Wilcox and de Sinéty fail to effect a careful analysis of these relations and a detailed criticism of their accounts will not be undertaken.

Otte expresses his belief in the genetic continuity of the chromosomes and describes a parasynapsis of spermatogonial derivatives in the first spermatocyte, and yet believes that there are two cross divisions without any reduction. Vejdovský, on the contrary, from studies upon other Locustidae, argues for a complete fusion of homologous spermatogonial chromosomes to form a new mixochromosome, which is later divided twice longitudinally. It is thus seen that the two recent workers upon Locustid material, who doubt the occurrence of a reduction division, both believe in the preservation of integrity of the chromosomes, but in one case through a double cross division and in the other through a double longitudinal cleavage.

Opposed to these few contradictory advocates of doubtful or impossible processes for the preservation of chromosome continuity are the large number who perceive and describe the only possible means for ensuring it—through a temporary union of homologous chromosomes and their later segregation in one of the spermatocyte divisions. While there are differences of opinion about the details of processes, there is agreement regarding the end results. Always in some form the opinion is expressed by the other students of Orthopteran spermatogenesis that because of their organization the chromosomes may be identified in the first spermatocyte as the descendants of the ones found in the preceding spermatogonium. All find a pseudoreduction which they ascribe to associations between the members of the diploid group. There is a constant specific number and there are recognizable, in most cases, either individual chromosomes, or groups of them, which may be traced from one cell generation to the next.

Criteria for such identifications are furnished by differences in size, form and behavior and they apply with great exactness

in the studies of different investigators, working upon similar materials secured from widely different sources, and through a great variety of technical methods. As a most marked instance of such concordance of results may be mentioned the accounts of spermatogenesis in *Stenobothrus*. This genus has been studied very generally and by cytologists of France, England, and America—Carnoy, de Sinéty, Girard, Meek, Davis—and yet the published accounts upon the same species show practical agreement upon the uniformity of number, sizes, forms and behavior of the chromosomes in different generations. If there were not genetic relations of the most exact character such agreements would be impossible. In a similar way, with the exception of the *Stenobothrus*-like species, and *Pamphagus*, the students of the *Acrididae* have reported a reduction of the 23 spermatogonial chromosomes to 12. Among this haploid number may be recognized the unpaired accessory chromosome and 11 paired elements whose members correspond in size, at least, with those of the previous generation.

Regarding the details of such a relation there is not a complete uniformity of opinion, but there is agreement upon the fact that by an association between definite members of the spermatogonial complex there are produced the bivalent structures of the first spermatocyte. For the *Acrididae* this is the opinion of Montgomery, Davis, Buchner, Brunelli, Sutton, Pinney, Nowlin, Robertson, Carothers and myself. Although the other families have been less studied these genetic relations appear to exist in the *Locustidae* according to Davis, Buchner, Stevens, and by both Otte and Vejdovský despite their views upon the character of the maturation divisions. For the *Gryllidae* Baumgartner and Payne; for the *Blattidae* Farmer and Moore, Stevens, and Wassilieff; and for the *Phasmidae* Jordan, are in agreement on the direct relation between the spermatogonial chromosomes and those of the first spermatocyte.

Of the greatest importance in determining the relations of chromosomes to each other in successive cell generations is the behavior of the accessory chromosome. There is almost uniform agreement regarding its direct and evident continuity, without

any loss of identity, from the spermatogonium over into the first spermatocyte. It was at first regarded as very different from the other chromosomes and was described as a nucleolus by many, but longer study has taught that it parallels the euchromosomes, although in a modified form, in every part of its history except in actual synapsis. That one of the spermatogonial complex, in most cases slightly distinguishable from its fellows throughout the 'division period,' should pass into the first spermatocyte without loss of form or identity and there perform much the same evolutions as the others, while still remaining distinctly delimited, is a very strong piece of evidence for the individual continuity of the others.

I believe therefore that it may fairly be said that the evidence from Orthopteran studies is strongly in favor of the view that the chromosomes of the last spermatogonial mitosis pass over as such into the first spermatocyte where they appear joined together in pairs. As I have stated elsewhere this seems to me a fundamental conception, for unless we are dealing with structures of definite and characteristic organization their manoeuvres in maturation are of small moment. In the further discussion it will therefore be assumed that the establishment of the concept of chromosome continuity has been made with a reasonable degree of certainty. This being done we have next to consider the method of association set up in the first spermatocyte chromosomes and the forms through which it is manifested.

b. Composition of chromosomes in the first spermatocyte prophase

A thoroughgoing consideration of the composition and form of the first spermatocyte chromosomes would require a full analysis of the complicated series of changes through which the chromosomes pass after the last spermatogonial division, when they enter into the tetrad condition. This cannot be done, for we are yet lacking a sufficient knowledge of these stages. We can but regard the haploid group of the first spermatocyte prophase and find therein the recognizable members of the unreduced complex, which, as I have shown elsewhere, may be accomplished very

definitely in many cases. The problem before us therefore, according to the terms of the argument set down, is to analyse the associated spermatogonial derivatives and to determine their relations as they prepare for, and undergo, division in the first spermatocyte.

In comparing the figures of the different investigators a striking uniformity in the outlines of the chromosomes is apparent. Also in the written descriptions there is always mention of the occurrence of chromosomes in the form of rods, crosses, rings, V's, X's, U's, etc. The practical uniformity of chromosome structure throughout the group seems to exist beyond question. Variation inheres therefore in the interpretations and not in the structures themselves.

It will suffice for the present comparison to begin a consideration of tetrad formation with the stage wherein the chromosomes have taken on their definitive condition and yet show their constituent parts. For convenience of discussion also the different chromosome forms will be taken up individually and the opinions of various authors regarding them compared.

1. Rod-shaped tetrads. Of all those appearing at this time the rod-shaped chromosome is the most simple, and, according to my view, is the fundamental type. It seems without doubt to be constituted of two spermatogonial chromosomes united end to end with their longitudinal clefts coincident and with their points of contact indicated by more or less divergence of their chromatids. This interpretation is given to the structure by Sutton, Montgomery, Davis, Buchner, Robertson, Pinney, Nowlin, Stevens and Wassilieff.

So far as the actual composition of the rod is concerned it is of no consequence whether there has been an earlier parasynapsis or whether, later, there is pre- or post-reduction. Should parasynapsis occur, however, there is a possibility that the plane of separation may be along the longitudinal cleft of the homologous elements instead of through the space between them. Extended rods in the metaphase would then be almost completely divided longitudinally at this time. Involved in this is also the

question of the position of fiber attachment, for, in case there should be such an early separation of the chromatids in this plane, the point of fiber attachment would still be at the synaptic ends, whereas if there were a segregation division at this time it would mean a shifting of the fiber attachment to the opposite ends.

It is not possible to come to a complete understanding of all these changes and it may be that each of these conditions is met in different forms. There are, however, a number of significant facts which point to the interpretation I have advocated from the first, i.e., of longitudinally split rods lying extended in the equatorial plate with the median synaptic ends directed toward the center of the plate and with fiber attachment at this point. One of these is the case of the unequal tetrad discovered by Wenrich in *Phrynotettix*, showing differential ends and dividing so that each daughter cell receives equivalent parts. Another is an also unpublished series of circumstances in *Trimerotropis*, worked out by Miss Carothers, showing the attachment of the so-called plasmasomes in such a way as to identify daughter chromatids. Here also the parts are recognized according to my description. The polar granules of Miss Pinney, the constancy of fiber attachment in *Stenobothrus*, the occurrence of cross-shaped chromosomes in the metaphase with one-half the separation accomplished, and finally the appearance in *Mecostethus* of a group of chromosomes, all but the smallest of which lie extended in the equatorial plate, form a mass of evidence which is most convincing when taken into consideration with that furnished by other chromosomal forms.

2. *V-shaped tetrads*. This form represents only a slight and unimportant modification of the simple rod. It is, as has been stated, such a chromosome bent at the synaptic point in the plane of the longitudinal cleft with more or less separation of the chromatids at the center. Such chromosomes appear in the late prophase and are easily recognizable in the polar view of the metaphase. They are shown in the figures of Sutton, Baumgartner, Davis, Nowlin, Granata, Payne and Robertson.

An occasional and interesting prophase modification of the V occurs when the chromatids separate along the longitudinal

split and at the same time show the space between homologous chromosomes. This produces a double V, the enclosed spaces of which are in planes perpendicular to each other. In such a structure it is not possible to distinguish these apart and it is theoretically possible that, in the subsequent concentration, either of these might represent the plane of division in the first spermatocyte. Such forms do not appear in the metaphase and they are comparatively rare in the prophase. In principle all the tetrads would be of this type if there were parasynapsis and equal separation of the chromatids.

3. *Cross-shaped tetrads.* This simple form of chromosome has been subjected to the most inexplicable misinterpretation possible, ranging from a practical denial of its presence by Wilcox, Otte, Vejdovský and Montgomery to the strange accounts of its formation by de Sinéty and Brunelli. It is correctly represented, in prophase by Stevens, Sutton, Davis, Otte and Robertson, and, in a modified form, due to the shortness of the chromatids, by Jordan. Failure to appreciate the true composition of this element can be due only to very imperfect or superficial observation. Nothing is clearer than the fact that the four arms, at the middle, lie in the same plane and that each is split along its length. Such appearances as are shown by de Sinéty in figures 123 and 124, and by Brunelli ('11) in figures 12 and 13 never occur, and Montgomery's supposed X-shaped chromosomes in figure 31 is in reality a V with the synaptic ends extended. Both de Sinéty and Brunelli are inexcusable for describing the cross as two superimposed chromosomes. Even the most casual inspection will show that the limbs lie in the same plane and have a clear diamond-shaped opening where their clefts intersect. Such figures are shown in the first spermatocyte of *Brachystola* by Sutton ('02, figs. 6 and 7); by Stevens ('05 in *Stenopelmatus*, figs. 56, 58, 59 and 64); by Robertson ('08 fig. 29); by Davis ('08, figs. 60, 166 to 168); by Nowlin ('08, figs. 3, 5 and 7, pl. 29, and figs. 3, 4, 13, 14, pl. 32); and by Carothers ('13, fig. 28).

4. *Ring-shaped tetrads.* Such tetrads I have always regarded as of the greatest significance, and I am now even more firmly of the opinion, if possible, that they present most clearly the real

composition of the first spermatocyte chromosomes. Objection has been made by Montgomery that they are complicated and difficult to interpret, but I do not believe that this is justified. He also states that they are transitory prophase stages which rarely persist into the metaphase. This is certainly an error, as Robertson has shown in the same genus studied by Montgomery, where there are always at least two in each metaphase complex and may be as many as seven. A fruitful source of error in determining the method of division in the ring chromosomes lies in the failure to discriminate between such rings as appear in *Hippiscus* and most other Acrididae, from those of *Stenobothrus*. In general it may be said that the mere structure of the ring is fairly interpreted, in most cases, but that its relation to the archoplasmic fibers and its actual division in the first spermatocyte mitosis have been much misunderstood. Because of its significance I shall make a careful analysis of this form of tetrad, as reported in the Orthoptera, and hope to make clear the cause for the confusion that has arisen.

That we deal here with a universal form is indicated by the fact that it is described and figured by Wilcox, Sutton, de Sinéty, Baumgartner, Montgomery (prophase), Farmer and Moore, Stevens, Moore and Arnold, Otte, Wassilieff (prophase), Brunelli, Pinney, Davis, Robertson, Gerard, Jordan, Morse, Granata, Meek, Nowlin, Hartman, Vejdovský, Carothers and myself. Indeed it may be said that practically without exception every investigator of recent years who has made a careful study of the maturation stages in the Orthoptera has seen and figured annular chromosomes. It would appear certain, therefore, that if a knowledge of the structure and division of such elements is attained we shall have made an important and significant advance toward a comprehensive understanding of all chromosomes in all the members of the order.

Essential to such an understanding is a knowledge of the exact structure of the ring in the prophase. This necessity I have always felt, and in my first paper on the Acrididae ('00, p. 94) appears this statement in italics: "Too much importance

cannot be laid upon the necessity for a thorough understanding of the early formative periods in the history of the first spermatocyte chromosomes." In my own work, and in that of my students, this stage has been carefully studied. It is nothing less than amusing therefore to read Brunelli's statement that I have not appreciated the real nature of the tetrads because of failure to study their formation in the prophase. On the contrary, it was just through such a study that I was saved from the errors of de Sinéty, Davis, Montgomery, Buchner and Granata in interpreting the ring chromosomes, as I hope to show.

The rings first appear clearly in the late prophase, following a period in which the chromatin is much diffused and almost unstainable. At this time the general forms of the chromosomes may be recognized in the hazy shapes within the nucleus, and among them are irregularly outlined rings. Very shortly after this, sometimes even in other regions of the same cyst, a central thread of darkly staining chromatin, split along its length, appears in each of the diffuse masses. Immediately one recognizes in the outlines thus established the various familiar rods, crosses, V's, rings, etc. Early in this period of their development the four chromatids of each chromosome lie approximately parallel and the rings are therefore much flattened, but with the rapid concentration of the hazy masses around their axial darkly staining threads, separation occurs and the rings spring open as if they were composed of elastic rods released from constraint. Such rings are shown in figures 99 to 105 and 114 to 123.

Misinterpretation begins here. As may be seen by inspection of figures 100, 115, 118 and 119, the rings are not plain bands but on one side there is an enlargement, and not infrequently there may be a corresponding one opposite this (fig. 122). When such structures are viewed laterally it is seen that each elevation from the ring is continuous with the half of the ring upon the same side as that on which it lies. When viewed *en face* (fig. 127) the ring appears as a cross, in which case the upper and lower arms are represented by the elevations which were seen in lateral view. From this it is clear that each lateral half of these elevations is

continuous with the quarter ring from which it arose. In other words, it may be stated that each prophase ring is divided into superimposed half rings by the longitudinal division of the conjoined homologous chromosomes, and that, by a plane at right angles to this, and passing through the points of endwise union of the homologous chromosomes, each half ring is divided into quarter rings. There is, then, no difference between a rod, V or ring except that in the latter the free ends are joined as at the opposite synaptic ends. Uniformity of construction is maintained. Such an interpretation of annular tetrads is given by Sutton ('02, figs. 5 to 7); Baumgartner ('04); Pinney ('08, figs. 18 a, d, e, f, h, 21); Davis ('08, figs. 59 to 61, 85, 170 to 178); Robertson ('08, figs. 26, 29); Buchner ('09, fig. 41); Granata ('10, figs. 27 to 29, text fig. 1 g, h, i, l, m, n, o, p).

A modification of this method of ring production consists in the simple approximation of the two longitudinally split chromosomes without divergence of their synaptic ends. This form of ring has been reported for the Acrididae only by de Sinéty and by Montgomery and their figures are of doubtful character. In the Locustidae, Otte and Vejdovský figure such rings along with others of the first type and it is probable that they overlooked small indications of divergence, for in my own studies of this family no marked differences from the Acrididae appeared.

Opposed to such a conception of ring formation is one in which the elevations just described as being constituted of diverging members of two superimposed half rings, are regarded merely as accidental crossings of the free ends of synaptic mates. No reason is assigned for the maintenance of this chance relation through the subsequent chromosome movements during which extensive adjustments take place which might reasonably be supposed to change or obliterate it. Doubtless such relations do occur in the prophase but upon close analysis it becomes very evident that they are not permanent and do not have a meaning opposed to that of the first interpretation. It is most significant that in all the figures drawn by those who hold to the second type not a single one represents a ring *en face*—the point of view from which the constitution of the ring is indubitable. I think it

may therefore be asserted beyond any reasonable doubt that the annular chromosomes of the first spermatocyte prophase are constituted as the great majority of investigators have described and figured them.

c. Relation of the chromosomes of the first spermatocyte metaphase to those of the prophase

There is little or no difference of opinion regarding the passage into the metaphase of the chromosome forms found in the prophase. With regard to the rings Montgomery states that although they are common in the prophase of *Syrbula* they occur but rarely in the metaphase. Robertson, however, showed clearly that this was an error. It may therefore be confidently asserted that the structural conditions shown by the prophase chromosomes are carried over into the metaphase, suffering only such modifications as result from continued condensation and possible chromatid movements.

d. History of the tetrads in the first spermatocyte mitosis

1. *Rod-shaped tetrads.* Reference has already been made to the position of the rod-shaped chromosomes in the first spermatocyte metaphase. That rods elongated in the spindle axis occur is not denied by any one; that they occur extended in the equatorial plate and divide in its plane is, however, disputed. My description of the actual occurrence of rods extended in the plane of the future cleavage and of their subsequent movements has been criticised by Montgomery, Davis and Gregoire as an assumption of complicated changes. The case of *Mecostethus* is a sufficient answer to any such criticism, for here nearly all the chromosomes lie in the equatorial plate and, since the fibers attach at the middle synaptic points, all the movements of division have to take place by an extension of the axial arms at the expense of the equatorial. Because of the unusual length of some of these chromosomes, however, instead of the chromatids remaining in contact entirely through this transformation, as usual, they sepa-

rate before the mid period of the movement and so appear as V's instead of as extended rods.

While *Mecostethus* presents an extreme case, it is not at all unique and many species show one or more of the same type of rods in metaphase. Wherever found they show a fiber attachment at the middle point and pass from the rod shape, through that of the cross, to the rod again, but now extended in the spindle axis and with an approximation of homologous chromatids instead of sister elements. This approximation is retained, in most cases, until the complete separation of the metaphase chromosomes, whereupon the chromatids spring apart and move toward the poles as V's. Obviously this is an equatorial division.

2. *The V-shaped tetrad.* As has already been stated the V-shaped chromosomes of the metaphase represents merely the rod type bent at the point of fiber attachment, so that the apex is directed toward the spindle axis. Such forms are rarely reproduced in the figures of investigators for the reason that polar views, from which point only they are visible, are not given. Attention has already been directed to this neglect. *Mecostethus* is the most striking instance of the occurrence of such chromosomes, but, as may be noted in plates accompanying this paper, they are common in *Hippiscus*, *Tropidolophus*, *Hesperotettix* and *Mestobregma*. Robertson shows in a series of complexes the forms of the individual chromosomes in the first spermatocyte metaphase, mainly in polar view, and here it is seen that the ones numbered 3, 4, 6, 7, 8 and 10 may appear as V's. If not in this form they are rings viewed laterally. V-shaped chromosomes may only with some difficulty be distinguished from rods or rings. *En face* they are again similar to these forms, and all of them resemble the crosses. These facts are very suggestive of the fundamental unity of form underlying all the diverse shapes present in the first spermatocyte. In actual division the V's are not to be distinguished from the rods.

3. *The cross-shaped tetrad.* While V's are a feature of the polar aspect of the metaphase figure, crosses appear only in lateral views, and then only when the chromosomes are regarded *en face*. While simple crosses occur, other chromosomes may present

this aspect when studied from the same position. Rings, rods and V's all look alike when viewed from the spindle axis in the equatorial plate. This is not a mere superficial resemblance but represents fundamental community of structure. In every case the cross is the result of the approximation of four chromatids in such a way that each chromatid is bent upon itself at right angles and so set that this angle is one of four grouped about a center. This center appears as a diamond-shaped opening, in favorable cases, but in the metaphase is usually apparently obliterated. The arms of the cross to which the fibers attach are approximately straight but the remaining two may be bent in any degree, even to the formation of rings.

Because the cross comes into view from the lateral aspect of the mitotic figure, it is common in the illustrations of many papers. De Sinéty ('01) shows such chromosomes in figures 87, 88, 110, 124, 131 and 137; Baumgartner ('04) in figures 13, 14, 16, 18 and 19; Moore and Arnold ('06) text figure; Pinney ('08) in figure 24; Buchner ('09) in figures 49, 51, 52 and 55; Granata in his text figures *d* and *e*; Nowlin ('08); ('13), figures 15, 18 of plate 29, figures 3 and 4 of plate 30, figures 25, 26 of plate 31, figures 19. of plate 32; Carothers ('13) in figures 29, 30, 31 and 47.

By most investigators these metaphase tetrads are represented objectively as they present themselves in stained preparations. Their interpretation depends upon a knowledge of their structure gained from the prophase, and by most students of the Orthoptera they are conceived to be the four armed figures with a cleft the length of each arm, apparent in the prophase but obscured by the concentration of the metaphase chromosomes. De Sinéty ('01), however, in his figure 124 a, represents a cross of two superimposed, independent chromosomes lying over each other at right angles. Similarly, Brunelli ('11) in figures 12 and 13 shows crosses thus constituted. Both of these authors unconsciously represent the cross correctly, de Sinéty in his figures 124 c, 131 and 137, Brunelli in his figure 14. The practical unanimity of opinion on this very clear question makes any further discussion of it unnecessary.

The movements of the chromatids, the relation of the spindle fibers to the chromosomes and the form of the anaphase chromosomes is similar to those of the rods and V's, so need not be considered here. The important matter in connection with the crosses is to understand that they represent a mid-stage in the movement of the chromatids, either in prophase or in metaphase.

4. *Ring-shaped tetrads.* If confusion exist in reports of the prophase condition of the rings, it is certainly multiplied when attempts are made to describe the metaphase forms. Here are added a number of factors which complicate the problem, but which yet give meaning to the chromosomes and to their movements. We approach an analysis of the metaphase conditions fortified by a reasonably strong consensus of opinion regarding (1) the relations of the chromosomes of this period to those of the spermatogonium, and (2) concerning the inner structure of the tetrads. Also (3) these are traced into the metaphase, where we have to note (4) their position in the mitotic figure, (5) the movements of the parts, (6) their relation to the archoplasmic fibers, (7) their character after separation. These will first be discussed for the Hippiscus type of rings and then for the Stenobothrus type.

(1) It is now universally admitted that the chromosomes of the first spermatocyte are made up of four chromatids, and a large number of cytologists believe that two of these are of maternal and two of paternal origin from the homologous pair of spermatogonial chromosomes. In the rings (2) they are so disposed that the maternal pair constitute one-half the periphery of the ring, the paternal pair the other, with the direction of the longitudinal cleavage passing through the arms of the ring parallel to the plane in which they lie, so as to produce two superimposed rings, alike in their derivation. (3) The metaphase sees these same forms placed in the mitotic figure where (4) they lie as rings extended in the equatorial plate, so that they are visible from the pole of the spindle. In most cases they lie perfectly flat in this plane so that the entire ring is sharply in focus at one time, with the exception of the synaptic extension, first seen in the prophase, which now lies at a point nearest the axis of the spindle and

shows as a darker spot at both high and low focus. Usually these two extensions fall within the width of the ring's wall and both are in the same vertical axis. Sometimes the annular body is more pointed at this inner side and the extensions may fall beyond the outer limits of the ring proper. Under such circumstances the enclosed space becomes drawn out in this direction also and passes somewhat into the extended wall of the ring (figs. 21 to 30, 129 to 133). Should the diameter of the curved rods be large in proportion to their length, the enclosed space is small, or may even disappear, and a cleft shows in the outer margin where non-synaptic ends are in contact (figs. 2, 3, 11). Viewed laterally or *en face* the annular chromosomes are with difficulty distinguishable from the V's and crosses (figs. 8, 9, 10, 25, 111), but oblique views are very clear and convincing (figs. 83, 133). (5) In division, during the spermatogonial mitoses, chromatids lying superimposed, are separated from each other, beginning at the inner end, where the fiber attaches, and moving in the same vertical plane to opposite poles of the spindle. *Mecostethus* shows exactly the same conditions in the first spermatocyte except for the fusion in pairs of homologous chromosomes. Ring-shaped chromosomes of the *Hippiscus* type maintain the same spatial relations in the cell and undergo similar movements of the chromatids.

(6) Of much importance is the fiber attachment for determining fixed points on the chromosomes. As has been shown, particularly in *Stenobothrus*, the point at which the fiber attaches remains the same from one generation of cells to the other. Where all the chromosomes are straight rods in the spermatogonium this point is always at the inner ends, and, in the first spermatocyte, the chromosomes occupy a similar position, as in *Mecostethus*, with the single exception that they are now joined in pairs. Where rings appear these relations are again preserved, only fusion has occurred at both ends of the univalent chromosomes. Terminal fiber attachment and rings of the *Hippiscus* type always occur together. (7) On separation all the anaphase chromosomes are simple V's and the former elements of the rings are no longer distinguishable from the other derivatives (figs. 5, 6, 7, 35).

Similar views with regard to the rings are held by Sutton, Pinney, Stevens, Robertson, Granata, Nowlin and Carothers. In the accounts and figures of these investigators is found substantial confirmation on each of the points of observation that I have just discussed. It is important to note here that among these are careful detailed studies of the rings in the prophase and representations of them not only in lateral view but also from above and *en face*. Such aspects of the rings are conspicuously lacking in the figures of those who favor the other explanation of ring formation and division. The numerous tabulated drawings of chromosome complexes in polar view by Robertson demonstrate the almost uniform occurrence of the rings in the equatorial plate—a circumstance that is not mentioned by the advocates of prereduction.

The second form of explanation of the behavior of the rings in the first spermatocyte was advanced by de Sinéty and was later endorsed by Montgomery, Davis, Buchner and Brunelli. It contradicts the one just discussed in these points: (a) The rings lie in the axis of the spindle and are visible on the margin of the complex in lateral view (de Sinéty, fig. 131; Buchner, fig. 55; Montgomery, fig. 23; Davis, figs. 63 and 64); (b) fiber attachment is opposite the synaptic ends (de Sinéty, figs. 123, 131; Buchner, fig. 55; Montgomery, fig. 33; Davis, fig. 63); (c) the synaptic ends are directed away from the spindle axis; (same figs. cited under b); (d) the plane of chromosome separation lies perpendicular to the equatorial plate (Sinéty, fig. 312; Montgomery, fig. 33); (e) whole univalent chromosomes separate from each other; (f) they pass across the equatorial plate and go to the pole on the opposite side (Sinéty, fig. 131; Buchner, fig. 55; Montgomery, figs. 32 and 33; Brunelli, figs. 14 and 15); (g) the diverging ends of the chromosomes to which the fibers attach do not lie superimposed but are displaced laterally (no views *en face* are given by any advocates of this method) and there would be no resemblance to the crosses or V's regarded from the same point of view. The two interpretations may fairly be contrasted by saying that, according to the first, uniformity of relations throughout the cell generations, and between elements of the complex, is preserved;

while according to the second diversity must rule under these circumstances. In one instance position of fiber attachment, relation to the spindle, position of daughter chromatids, and method of division remain the same; in the other, these are subject to variation in greater or less degree. It will not be necessary to consider at length the disagreements between the views, for, in presenting my interpretation, I have already gone into these matters. There are, however, some specific objections which I should like to urge against the evidence presented on the various points which I have distinguished as characteristically different in the second account, and these I will take up in order.

a. Position of ring in the first spermatocyte metaphase. Sinéty represented this (fig. 131) as being so placed that the central opening becomes visible in lateral view. In this he was followed by Davis and Buchner, while Montgomery and Brunelli fail to show exactly such appearances, but represent extended chromosomes with their inner ends being pulled past each other. Davis illustrated his interpretation by rope models very clearly. To one familiar with the conditions it is apparent that what de Sinéty and Davis saw were oblique views of rings near the end of their division, while Montgomery and Brunelli were confronted by lateral, edgewise views of rings which they failed to interpret as such, definitely, because they had not sufficiently studied polar views. I believe this criticism is justified for the polar aspect of the chromosomal complex is so striking and so illuminating that if it had been appreciated it would have been figured. (Brunelli shows in fig. 13 a complex before the full establishment of the equatorial plate.) Few or no complete complexes are represented by these authors—a striking contrast to the large series pictured by Robertson. To some extent also this criticism lodges against Davis and Buchner. The former represents no polar views in his carefully prepared plates and only two in outline text figures while Buchner shows but one, in which no attempt is made to indicate the structure of the chromosomes. A mere inspection of the numerous drawings and photographs given in this paper will be sufficient to show

that the rings really lie in the plane of the equatorial plate and not parallel to the spindle axis.

b. Fiber attachment. This is always nearest the axis of the spindle, therefore if it be at the free ends of synaptic mates the point of union must be directed away from the spindle. This means either that the fiber attachment has shifted to the opposite end from that in the spermatogonium, or that synapsis has been at the distal ends. The evidence seems to point strongly to fixity of fiber attachment, and if whole spermatogonial chromosomes were separated in the first spermatocyte, synapsis would have to occur, or at least persist, longest at the distal ends. Either assumption calls for differences between the rings and the other forms of tetrads instead of conformity with them.

c. Position of synaptic ends. If fiber attachment is at the free ends of synaptic pairs then of necessity the fused ends must be directed away from the spindle. This is just the opposite of the conditions which certainly prevail in the case of *Mecostethus* and all other forms with V-shaped tetrads. Not only the prophase structure of the rings but all other considerations of order in the behavior of the chromosomes speak against this view. It should be noted that neither Davis nor Buchner, who figure the rings with divergent cross-shaped synaptic ends in the prophase, show this condition at the outer extremity of the rings in the metaphase. Here again is diversity conceived in place of uniformity.

d. Relation of division plane to equatorial plate. In the division of chromosomes, planes of cleavage lie within that of the equatorial plate, at least at the inner end where are attached the archoplasmic fibers. By the interpretation of de Sinéty and his followers this is not true, for the chromosomes are pulled past each other in the plane of the spindle axis. This is not, in fact, a division of the chromosomes in the proper sense of the term, but is a mere disjunction, but one step removed from the exploded 'primary type' of Goldschmidt. It is to be noted here that in the case of *Stenobothrus*, where a segregation division occurs, the rings show extensive movements of the chromatids during the metaphase. Divergence from normal behavior is again involved in this explanation.

e. Movements of chromatids. Whole univalent chromosomes are separated bodily by this form of division, according to the explanation of Montgomery, Davis and Buchner, but because of his conception of a double longitudinal division, de Sinéty sees in it an equational division. The evidence against this has already been given and will not be repeated here.

f. Relation of chromosomes to spindle poles. According to de Sinéty and those who follow him, there is described a most unique movement of the chromosomes involved in the ring. Instead of passing to the spindle pole toward which they lie, they cross over to the opposite side. All explanations of cell division, which involve an element of organization finding expression in the bipolar condition of the cell parts at this time, are controverted by such a process. I feel convinced that there is no such condition of the chromosomes and I do not believe that the reported twisting of the chromosomes in the metaphase described for many forms, particularly plants, is correct. As a pure matter of observation I am sure that it does not occur in the Orthoptera. Perhaps the appearance of the looped or double ring chromosomes in many species of this order has been responsible for the error. By the interpretation of de Sinéty (figs. 124 and 125), Brunelli (fig. 15), Buchner (fig. 42), Davis (figs. 184 and 185) these are merely twisted rings. Although occasionally such conditions are met in the prophase the forms that later appear in the metaphase are not thus constituted. At this time the second ring, the one further removed from the center of the equatorial plate, will always be found to lie perpendicular to the primary ring and to merge symmetrically into it. If these were really twisted rings they would commonly lie more nearly in the same plane and the outer loop would not enter into the inner symmetrically. When prophase structures, corresponding to those of the metaphase, are studied it is found that the secondary ring is really produced by the further development, at the contrasynaptic ends, of occasional divergences of the chromatids of the same character as the ones found commonly at the synaptic ends; and these may even go so far as to start the production of a third ring in the same plane as the primary one. In other words, the chromatids do

not cross from one side to the other but remain constantly superimposed. These conditions have been clearly worked out by my students and myself on all the forms studied, but have not generally been understood. An exception to this statement is furnished by Granata who in his figure 28 and text figure 1, *o*, (fig. 141) shows accurately the structure of the looped chromosomes. All considerations of uniformity in chromatid structure and movement speak against the transposition of structures to the opposite pole of the spindle. Boveri has fully discussed this matter in relation to *Ascaris* chromosomes.

g. Relation of rings to crosses. Crossed ends of chromosomes when viewed across the equatorial plate, *en face*, would necessarily appear displaced laterally—they would not be superimposed in one plane. Despite the number who have advocated this relation not one has shown a figure of a chromosome in such a position. The omission is significant, for appearances of this kind are not to be found. Such an aspect of the ring is a crucial test of de Sinéty's conception. From this point of view, indeed, the rings are of the same appearance as crosses, or, viewed somewhat obliquely, show clearly the extended ends directly over each other and with the lateral parts of the annulus merging symmetrically into each (figs. 130, 133).

So far as the prophase is concerned, the rings in *Stenobothrus*, *Chorthippus*, *Chloëaltis* and the unidentified species which I have figured—which I shall speak of as the *Stenobothrus* type—do not seem to show any differences from the ones found in *Hippiscus* and other similar forms. In the metaphase, however, it is clear that they bear a different relation to the archoplasmic fibers; and, by comparison with the chromosomes of the spermatogonium and second spermatocyte, it appears that this is a constant feature of the organization of certain ones of the complex. This is made sure by the work of all investigators who have studied *Stenobothrus*, but was emphasized by Davis. A failure to appreciate the difference between the rings of *Stenobothrus* and *Hippiscus* types has been responsible for much of the confusion that exists in the literature regarding Orthopteran spermatogenesis. It is very necessary

that this be removed because the *Stenobothrus* form of chromosome, while rare in the Orthoptera, is very common in other animals and in plants. Had these matters been understood many extended reviews, seeking to reduce all maturation phenomena to a type, upon the basis of chromatid relations in the first spermatocyte metaphase, would not have been written. It is barely possible that a complete understanding of synapsis conditions may result in such a harmony, but with our present knowledge of the subject the metaphase relations are very different and point almost certainly to prereduction in *Stenobothrus* rings and to post reduction in those of *Hippiscus*. The difference between these two types of annular chromosomes relate to the fiber attachment and to the position in the spindle, the latter being a consequence of the former. By some chance there is no difference of opinion on these matters, and all observers agree that in *Stenobothrus* the rings lie extended in the axis of the spindle with fibers attaching somewhere near the middle of each of the half rings that later enter the anaphase. There results accordingly a reduction division.

It is difficult to see how these sharply contrasting conditions could be confused, yet neither de Sinéty nor Davis, both of whom studied *Hippiscus*-like chromosomes, differentiated between the two varieties of rings. That this was due entirely to misinterpretation and not to varied conditions is rendered certain by comparing two adjoining figures of de Sinéty, figure 125 showing the *Stenobothrus* ring extended along the spindle with subterminal fiber attachment, and figure 132 of *Oedipoda*, the only polar view, with the rings lying in the equatorial plate with terminal attachment of fibers. Davis also shows clear lateral views of the spindle of *Stenobothrus* with extended rings (figs. 88, 91) and in text figure N sketches of two cells in polar view with rings showing. In both instances mistakes in determining the correct relation of the *Hippiscus* rings to the archoplasmic fibers resulted in an apparent uniformity.

Stevens ('12) shows rings of this type in *Ceuthophilus*, Jordan ('08) in *Aplopus*.

Summarizing the comparison between the rings of *Hippiscus* and of *Stenobothrus* under the various headings used in the consideration of this structure we find that there are in each case (1) four chromatids derived two from the male parent and two from the female parent, (2) joined so that homologous pairs are united end to end and sister pairs are superimposed. (3) These relations of the prophase are carried over into the metaphase where (4) in *Hippiscus* the rings lie in the equatorial plate with sister chromatids directed toward opposite poles of the spindle, while in *Stenobothrus* although the parts of the tetrads are in the same relation to each other the whole chromosomes lie extended in the direction of the spindle axis with sister chromatids toward the same pole. (5) In *Hippiscus* the sister chromatids glide away from each other and become extended in the line of the archoplasmic fiber beside homologous chromatids, thus progressively reducing the size of the ring to supply the increased length of the extensions; in *Stenobothrus*, on the contrary the whole rings separate directly into half rings or V's. (6) The rings in *Hippiscus* have the fiber attachment at the synaptic ends, which are slightly drawn out, directly above each other, and which consist each of homologous chromatids, while in *Stenobothrus* the fiber attachment is approximately median and in each case involves connection with sister chromatids. (7) The dyads of *Hippiscus* in the anaphase are simple V's like all the others of the complex, while the ones in *Stenobothrus* are double V's and unlike the simple V's of the metaphase rods in the same complex. (8) The results of the separation of the rings of *Hippiscus* is an equational division while in *Stenobothrus* it is a reductional one.

e. The chromosomes in the second spermatocyte

There is comparatively little difference of opinion regarding the actual appearance of the chromosomes in this generation, and, so far as interpretation is concerned, it is altogether dependent upon the meaning attached to the first spermatocyte division. While therefore extended discussion is not called for, it will be convenient to keep separate the consideration of the chromosomes of *Hippiscus* and of *Stenobothrus* types.

1. *Hippiscus* type. The striking variation of form appearing among the metaphase chromosomes of the first spermatocyte gives way in the second spermatocyte to as marked a uniformity. The paired, straight rods of the first spermatocyte anaphase reappear in the succeeding metaphase in the same sizes and forms, and between these two stages—through the telophase and prophase—they may often be recognized individually. This visible continuity is a variable matter in different species, but almost always the individual elements may be traced without confusion. Here, as elsewhere, the accessory chromosome comes into prominence during the prophase by reason of its greater density, clearly indicating the cells wherein it lies. To some extent, however, it shows a loosened structure like the euchromosomes and again in this way presents a parallelism with them. The changes between the two generations of spermatocytes take place rapidly, and occasionally cysts are formed in which appear telophases of the first spermatocyte and metaphases of the second spermatocyte. It is evident that no profound changes are accomplished at this time—everything points to a rapid rearrangement of formed chromosomes into a new division figure with the least possible change or movement of the cell elements.

A study of the metaphase figure discloses physical conditions very similar to those of the spermatogonia. The chromosomes are straight rods arranged radially, for the most part, about the spindle axis with fiber attachment at the inner ends (figs. 16 to 19, 31, 49 to 58). The same series of sizes apparent in the earlier generation again presents itself, but now in a single instead of a duplicate grouping. Presence or absence of the accessory chromosome marks the two classes of cells descended from each secondary spermatogonium. One notable difference between the chromosomes of the spermatogonia and of the second spermatocytes manifests itself. During the earlier period the chromosomes were much extended and the chromatids were closely apposed, while in the second spermatocyte the chromosomes are shorter and show a marked divergence of their halves. This tendency of the chromatids to separate may go to the extreme of extension in a straight line if the chromosome happens to lie toward the

center of the equatorial plate (fig. 57). Only at the point of fiber attachment do the chromatids remain in contact. Separation of the chromosome halves already exists at this time, and movement to the spindle poles as simple, straight rods easily follows. The accessory chromosome, being also a dyad, divides like the other chromosomes. Upon these points there is practical agreement by all who have studied the Acrididae, so a further discussion is not called for; but whether the two chromatids are sister elements or merely homologues is a mooted question. It entirely depends upon what has happened in the first spermatocyte and the evidence of the earlier generation is much clearer. According to my belief, in most of the chromosomes they are homologous elements and are separated, through a segregation^{*} division by this mitosis.

2. *Stenobothrus* type. In this, as in the *Hippiscus* type, the sizes of chromosomes in the spermatogonium reappear in the second spermatocyte but, in addition to this, also the forms recognizable in the earlier generation are found. Indeed, aside from the haploid condition of the chromosome group, all the relations of the spermatogonium are preserved. As may be seen from figures 68 to 76, there are, in the *Stenobothrus*-like form I have studied, always six *Hippiscus*-like rods in each second spermatocyte and four J- or V-shaped dyads. When the accessory chromosome is present there is added a fifth chromosome with non-terminal fiber attachment. In *Stenobothrus curtippennis* Davis reports a similar constancy of forms and his conclusions are borne out by the work of Meek and supported by the less detailed studies of de Sinéty and Gerard. It seems established beyond question that organization is so exact that even the form of the chromosomes is

^{*} In this paper and others I have used the term 'segregation division' synonymously with 'reduction division.' It seems to me a preferable term with our present knowledge of cellular processes, since it indicates the nature of the separation in concordance with our understanding of the composition of the tetrads out of elements derived from the two parents. There is no 'reduction' in Weismann's sense, only a segregation of paternal and maternal derivatives. Of course this involves the conception of such a biparental composition of the tetrads, but practically the same idea is at the bottom of any 'reduction' scheme and so it would not hold more against one than the other.

repeated in every cell of every individual of a species, and, in the case of *Stenobothrus*, in every species of the genus. Further, this question of form finds its explanation in the relation which each chromosome bears to the archoplasm of the cell—it represents a fundamental matter of cellular organization.

Apparently, in interkineses, all this is lost, but the history of the *Stenobothrus* chromosomes shows that this is not so. The forms of the spermatogonial chromosomes reappear in the grand-daughter cells, and in these two derivatives may be found all the chromosomes of the single cell of the earlier generation, each recognizable by its size and form. This is the common experience of different investigators working upon material derived from the most remote sources and from specimens classified into four or more species. Since the actual act of reproduction is seen in each mitosis, the only conclusion that can be drawn is that these chromosomes of certain definite sizes and forms possess these attributes in the second spermatocytes for the reason that they are lineal descendants of the like series in the spermatogonia. It is justifiable to conclude further that the like series in various species of the genus is the result of the same laws operating over a longer period of time. The animals now in existence within this genus are here because they descended from similar organisms in the past, and their common characters are due to their common ancestry: the chromosomes within the cells of these animals bear the stamp of resemblance in series for the same reason. Indeed the processes of reproduction in the greater and in the less are coincident and form a common problem.

It causes no confusion therefore to find interposed between the two cell generations with similar formed chromosomes, an intermediate one in which these configurations are not present, for the index of these variations is furnished by the position of the fiber attachment. In each case it is possible to identify the particular element by this feature of its organization, in connection with its relative size in the complex. We are just beginning to understand something of the exactness of chromosome organization, and the conditions in *Stenobothrus* furnish one of the most generally recognized cases of it. Attention has been directed

to the very obvious conditions in the formed chromosomes of the metaphase, but more extensive knowledge must come from an intimate analysis of structure, which can only be made when the elements exhibit their finer details within the nuclear membrane. What has already been accomplished in this way lends great encouragement to the belief that we may expect to know much more about the structures, relations and functions of the chromosomes than we do at the present time. It may justly be said here that the evidence for persistent individuality afforded by the relations between the second spermatocyte chromosomes and those of the spermatogonia is strengthened and made more exact the further the study is pushed back into the entire history of the chromosomes.

According to many investigators, the result of the second spermatocyte mitosis is to separate daughter chromatids through an equational division. So far as the chromosomes with median or subterminal fiber attachment are concerned, I am inclined to agree, although it must be admitted that with parasynapsis the opposite might be the case. Chromosomes with terminal fiber attachment, however, show the same relations and behavior as similar ones do in *Hippiscus*. Figures 64, e, f, show such elements extended in the equatorial plate and they must divide along their length if at all. The evidence in support of this view is stronger in *Stenobothrus* than elsewhere, for the reason that the position of fiber attachment demonstrably remains constant. If this be the case then in such chromosomes as the one shown in figure 64, e, lying in the equatorial plate, the synaptic ends are also the ones to which the fibers attach. Division must separate daughter chromatids from each other, along the longitudinal split of the chromosomes. Beside this chromosome, in the same complex, appears a rod-shaped chromosome extended in the direction of the spindle axis. This I conceive to have already passed through the changes which its neighbor is just beginning. Under any other assumption it must be that synapsis has taken place at the distal ends of the chromosomes or that fiber attachment has shifted after synapsis. The evidence is all in favor of constancy in position of fiber attachment, and actual movement

of chromatids is shown in cross-shaped chromosomes such as are found in many species including the *Stenobothrus*-like form I have studied (fig. 64, f).

The result of the second spermatocyte mitosis is therefore neither entirely equational or segregative, for certain ones of the chromosome complex are separated in one way, others in the opposite way.

SPECIES OF ORTHOPTERA WHOSE SPERMATOGENESIS HAS BEEN DESCRIBED

1. *Acrididae*

- Artom, C. ('09) *Stauronotus*
 Brunelli, G. ('10) and ('11) *Tryxalis nasuta*
 Buchner, P. ('09) *Hippiscus* sp.
 Bütchli, O. ('71) *Acridia*
 Carothers, E. ('13) *Brachystola magna*, *Arphia simplex*, *Trimerotropis suffusa*, *T. schaefferi*, *T. agrestis*, *T. maritima*
 Carnoy, J. B. ('85) *Oedipoda coerulea*, *Acridium lineola*, *Stenobothrus viridulus* or *bicolor*
 Davis, H. S. ('08) *Arphia tenebrosa*
 Chortophaga viridifasciata
 Dissosteira carolina
 Hippiscus tuberculatus
 Stenobothrus curtippennis
 Gerard, Pol ('09) *Stenobothrus biguttulus*
 Giglio-Tos, S. ('08) *Pamphagus marmoratus*
 Granata, L. ('10) *Pamphagus marmoratus*
 Hartman, Frank ('13) *Schistocerca alutacea*
 McClung, C. E. ('00) *Hippiscus phoenicopterus*
 ('05) *Hesperottetix speciosus*
 Hesperottetix viridis
 Hesperottetix pratensis
 Mermiria bivittata
 Chortophaga viridifasciata
 Meek, C. F. U. ('11) *Stenobothrus viridulus*
 ('12a) *Stenobothrus viridulus*. *S. bicolor*, *S. parallelus*
 ('12b) *Stenobothrus curtippennis*
 Stenobothrus parallelus
 Stenobothrus viridulus
 Stenobothrus bicolor
 Minot, C. S. ('80) *Melanoplus femur rubrum*
 Montgomery, T. H. ('05) *Syrbula acuticornis*
 Nowlin, Nadine ('08) *Melanoplus bivittatus*
 Pinney, Edith ('08) *Phrynotettix magnus*
 Robertson, W. R. B. ('08) *Syrbula admirabilis*

de Sinéty, R. ('01) *Oedipoda miniata*

Stenobothrus parallelus

Veselý, J. ('13) *Chrysochraon dispar*

Sutton, W. S. ('00) ('02) *Brachystola magna*

Wenrich, D. H. — *Phrynotettix magnus*

Wilcox, E. V. *Melanoplus femur rubrum*

2. *Locustidae*

Buchner, P. ('09) *Decticus verrucosus*

Davis, H. S. ('08) *Steiroxys trilineata*

Gilson, G. ('84) *Locusta viridissima*

Decticus verrucivorus

McClung, C. E. ('99) *Xiphidium fasciatum*

('02) *Anabrus*

Orchesticus

Scudderia

Microcentrum

Meek, C. F. U. ('12) *Steiroxys trilineata*

Minot, C. S. ('86) *Anabrus*

Otte, H. ('96) *Locusta viridissima*

Sabatier, A. ('90) *Locusta*

Schellenberg, A. ('13) *Diestramena marmorata*

de Sinéty, R. ('01) *Orphania denticauda* C.

Platycoleis grisea Fab.

Stevens, N. M. ('05) *Stenopelmatus*

('12) *Ceuthophilus*

Veselý, J. ('13) *Locusta viridissima*

Vejdovský, F. ('12) *Decticus verrucivorus*

Diestramena marmorata

3. *Gryllidae*

Baumgartner, W. J. ('02) *Gryllus assimilus*

('04) *Gryllus domesticus*

Brunelli, G. ('09) *Gryllus desertus*

Buchner, P. ('09) *Gryllus campestris*

Carnoy, J. B. ('85) *Gryllotalpa vulgaris*

Guthers, S. ('06) *Gryllus domesticus*

Meek, C. F. U. ('08) ('12) *Gryllus domesticus*

Payne, F. ('12) *Gryllotalpa borealis*

vom Rath, O. ('91) ('92) *Gryllotalpa vulgaris*

de Sinéty, R. ('01) *Gryllus domesticus*, *Nemobius sylvestris*

Voinov, D. N. ('04) *Gryllus campestris*

4. *Blattidae*

Carnoy, J. B. ('85) *Blatta germanica*

Periplaneta orientalis

Erlanger, R. v. ('97) *Blatta germanica*

Moore, J. E. S., and Robinson, L. E. ('05) *Periplaneta americana*

Morse, Max ('09) *Blatta germanica*

Periplaneta americana

Leucophaea maderiae

Stylopyga orientalis

Wassilieff, A. ('07) *Blatta germanica*

5. *Phasmidae*

Carnoy, J. B. ('85) *Bacillus linearis*

Jordan, H. E. ('08) *Aplopus mayeri*

de Sinéty, R. ('01) *Leptynia attenuata*

Menexenus obtusespinosus

Dixippus morosus

6. *Forficulidae*

Carnoy, J. B. ('85) *Forficula auricularia*

Meek, C. F. U. ('12) *Forficula auricularia*

Randolph, H. ('08) *Anisolabis maritima*

St. George, v. la V. ('87) *Forficula auricularia*

Stevens, N. M. ('10) *Forficula auricularia*

de Sinéty, R. ('01) *Forficula auricularia*

Labidura riparia

Zweiger, H. ('06) *Forficula auricularia*

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EXPLANATION OF PLATES

Figures are drawn with a camera lucida at an original magnification of 2860 diameters, which, in reproduction, is reduced to 1800. The photomicrographs were made with a Zeiss apochromatic 2 mm. N. A. 1.40, projection ocular 4 and Watson 'Parachromatic' oil immersion condenser. The original magnifications were 600 and 1000 diameters which are here reduced to 525 and 875 respectively

PLATE 1

EXPLANATION OF FIGURES

1 to 19 Spermatocyte chromosome complexes from *Hippiscus*.

1 to 4 Polar views of first spermatocyte metaphase, with varying number of rings. The accessory chromosome, marked X, appears within the outer circle of chromosomes and is rough in outline.

5 and 6 Daughter groups in first spermatocyte anaphase. Former associated members of tetrads are seen in the same relative positions in the two groups. In figure 6 the accessory chromosome, X, appears, for which there is no mate in the opposite group.

7 Lateral view of an early first spermatocyte anaphase. In figures 5, 6 and 7 the dyad character of the anaphase chromosome appears clearly.

8 to 10 Lateral views of the first spermatocyte metaphase complexes, in which the members have been extended in rows with homologous elements in the same vertical column and with the smaller chromosomes at the left and the larger of the series at the right. The variable shape of the same element at different stages of division is shown.

12, 13, 16 to 19 Polar views of second spermatocyte metaphase chromosome complexes. The accessory chromosome, X, is present in figures 12, 16, 17 and 19. In figure 17 it is shown surrounded by a vesicle.

14 and 15 Somewhat oblique lateral views of first spermatocyte metaphase.

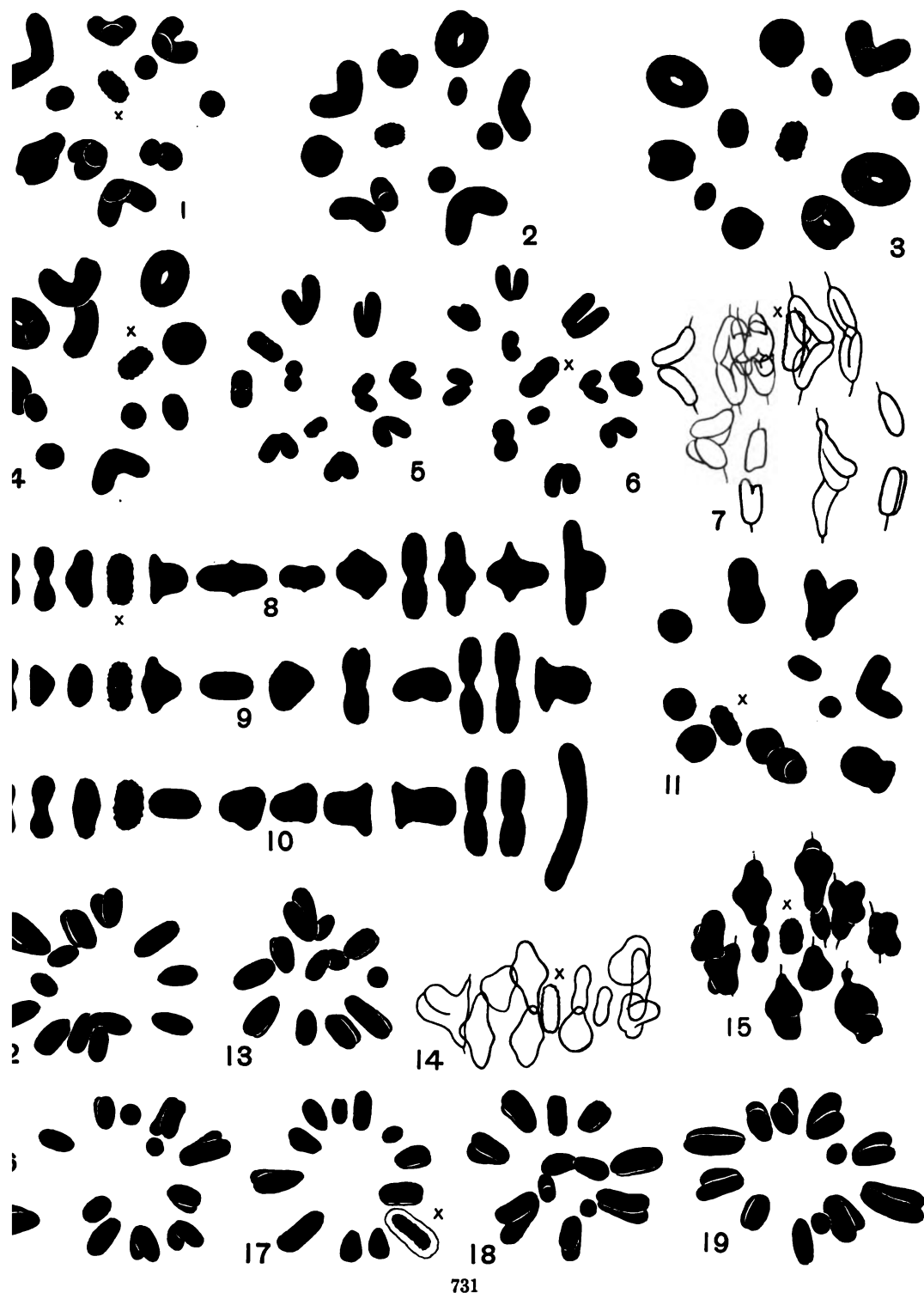


PLATE 2

EXPLANATION OF FIGURES

20 to 31 From *Tropidolophus*.

20 Polar view of spermatogonial complex.

21 to 24 Polar views of metaphase groups in first spermatocyte. The accessory chromosome is centrally placed and is irregular in outline, as in *Hippiscus*.

25 Lateral view of first spermatocyte metaphase complex with the members extended in rows. At *a* is a ring, *en face*; at *b* a lateral view of a ring with the contrasynaptic ends also drawn out.

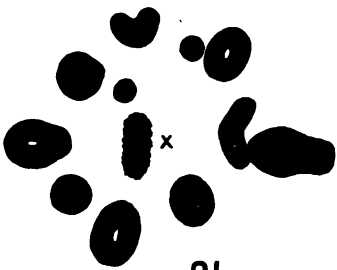
26 to 30 Polar views of first spermatocyte metaphase complexes.

29 A complex, six members of which are plain rings. A photomicrograph of this group is shown in figure 110 and in figure 140 appears a photograph of wax models of the group arranged as in the cell. Similar rings are seen in the remaining polar views, but in addition there are others, evidently rings, which are not circular in outline but show various enlargements on the other side. These modifications are due either to a simple divergence of the contrasynaptic ends or to a further extension of these so as to complete a secondary ring at right angles to the first.

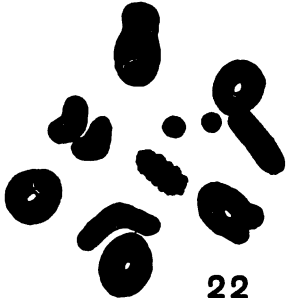
31 Polar view of second spermatocyte metaphase with a complex of 12 chromosomes. The uniformity in shape of the chromosomes in this cell generation is well shown.



20



21



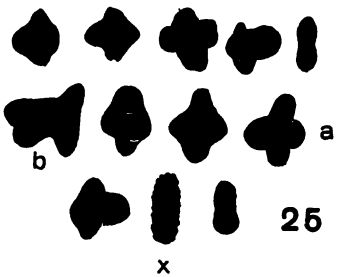
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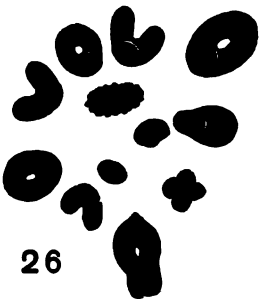
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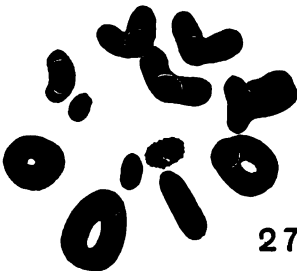
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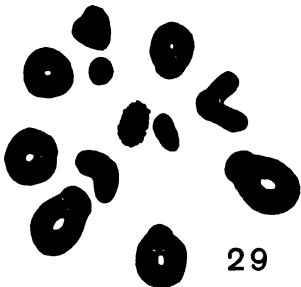
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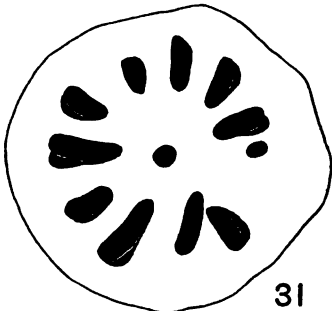
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29



30



31

PLATE 3

EXPLANATION OF FIGURES

32 to 58 From *Mecostethus lineatus*.

32 to 34 Polar views of first spermatocyte metaphase complexes. All the chromosomes are in the form of rods, straight or, more commonly, bent at the synaptic ends into V-shaped figures. The entire absence of the common rings is noteworthy in this species (see fig. 10% for photomicrograph of the cell drawn in fig. 34).

35 Beginning of the first spermatocyte anaphase. The chromosomes here are all of the common V-shape, the same as in complexes with rings (cf. figs. 5-7). The only unusual behavior is the divergence of the limbs of the V's before separation of daughter chromatids in the equatorial plate.

36 Oblique view of three euchromosomes and the accessory chromosome in the first spermatocyte metaphase.

37 Polar view of the first spermatocyte metaphase.

38 Various metaphase chromosomes of first spermatocyte (except the lowest chromosome which is late prophase). The character of the chromosomes at this period is shown in the different views.

39 Four first spermatocyte metaphase euchromosomes. The chromatids of the two small euchromosomes remain in contact until the end of the metaphase as in most other species.

40 Oblique view of three euchromosomes in early anaphase.

41 to 43 Polar views of first spermatocyte metaphase. Twelve is the haploid number, but because of the early poleward movement of the accessory chromosome it does not often appear in the plate. One other chromosome is missing from figure 37.

44 Polar view of spermatogonial complex in metaphase.

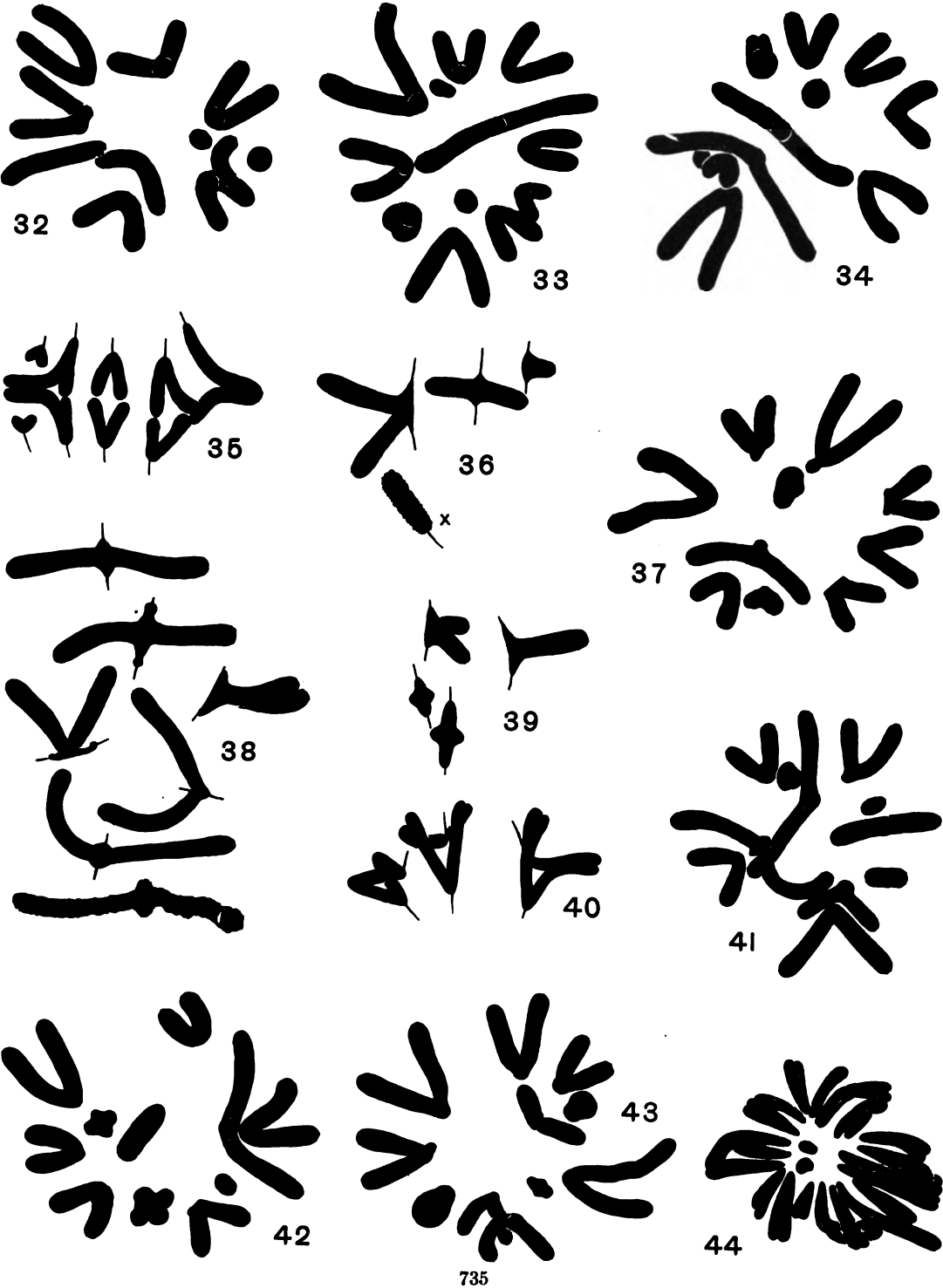


PLATE 4

EXPLANATION OF FIGURES

45 Lateral view of four euchromosomes and the accessory chromosome in first spermatocyte metaphase.

46 Polar view of first spermatocyte metaphase.

47 to 58 Second spermatocyte metaphase complexes.

47 Polar view with the accessory chromosome at the center of the group. The series of sizes is well shown, but it is worthy of note that all the members are only approximately two-thirds the size they were in the first spermatocyte.

48 Lateral view showing superposition of the chromatids and their wide divergence.

49 and 50 Polar views.

51 and 52 Lateral views.

53 to 58 Polar views; in figures 47, 50, 53, and 58 twelve chromosomes appear, in the others the accessory chromosome is lacking.

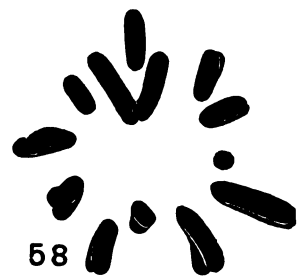
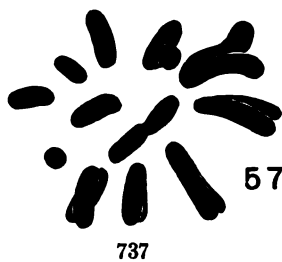
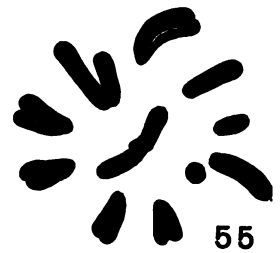
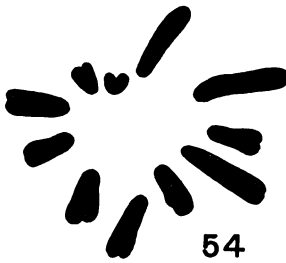
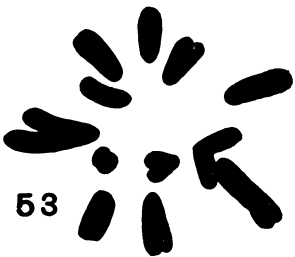
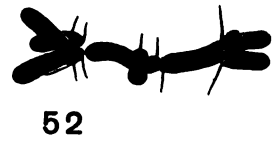
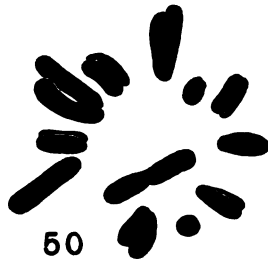
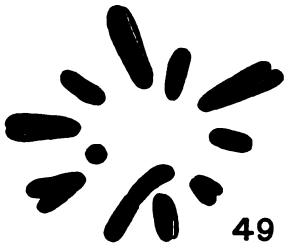
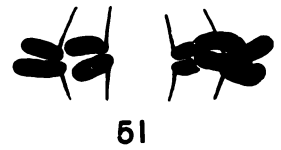
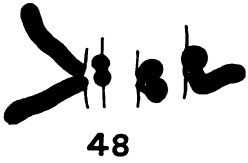
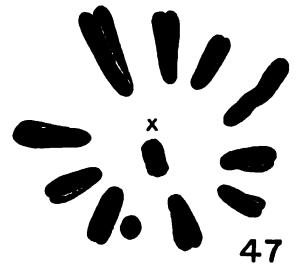
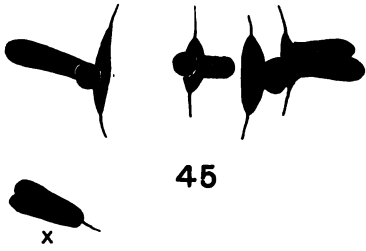


PLATE 5

EXPLANATION OF FIGURES

59 to 76 From the unidentified *Stenobothrus*-like form described in the text.

59 to 62 Polar views of spermatogonial metaphase. Of the twenty-one chromosomes (fig. 60 shows but twenty) twelve are the common rod-like forms with terminal attachment and the remaining nine have either median or submedian attachments.

63 Lateral view of first spermatocyte metaphase showing four ordinary shaped tetrads, a ring placed in the plane of the spindle axis, and, at the left, another such an element, but with the synaptic ends drawn out and bent to form a ring in the plane of the equatorial plate.

64 Various first spermatocyte chromosomes in metaphase or early anaphase. Tetrads formed from spermatogonial chromosomes with non-terminal fiber attachment shown at *a*, *b*, *c*, *d*, *g*, and *h*. At *e* and *f* are others produced by union of spermatogonial chromosomes with terminal fiber attachment.

65 Lateral view of the first spermatocyte metaphase showing four chromosomes of ordinary type and two with non-terminal fiber attachment.

66 Lateral view showing conditions as in figure 65 and the accessory chromosome which has a median attachment instead of terminal as in most species.

67 Polar view of first spermatocyte anaphase.

68 to 76 Polar views of second spermatocyte metaphase. The same series of sizes and forms apparent in the spermatogonium may now be seen in the grand-daughter cells.

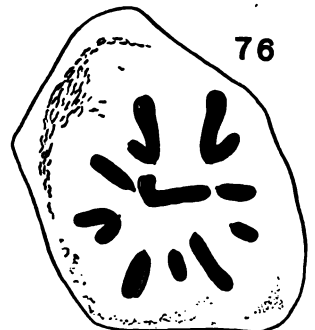
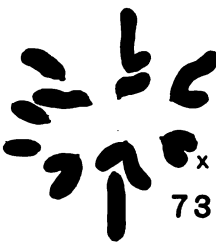
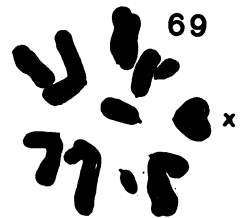
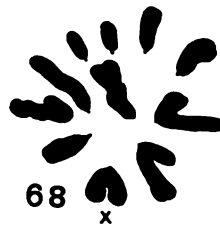
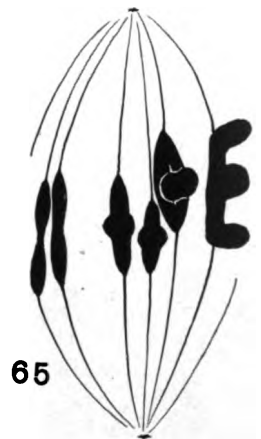
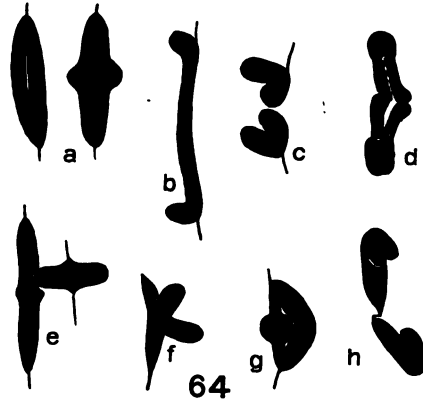
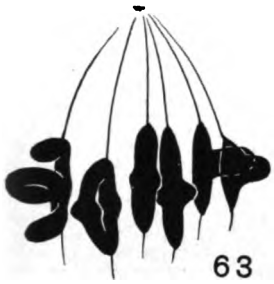


PLATE 6

EXPLANATION OF FIGURES

77 Polar view of spermatogonial metaphase of *Hesperotettix speciosus*. There are twenty-one rod-shaped chromosomes and a V with unequal arms. The subsequent history shows this to be a multiple chromosome consisting of the accessory chromosome and one euchromosome. While of the same shape as the chromosomes of *Stenobothrus* which have subterminal attachment it is of different composition.

78 Lateral view of a first spermatocyte metaphase complex of *Hesperotettix speciosus*, the multiple chromosome at the right with the accessory chromosome portion marked X.

79 Two multiple chromosomes from *Hesperotettix speciosus*.

80 Two multiple chromosomes from *Hesperotettix viridis*.

81 Two multiple chromosomes from *Hesperotettix pratensis*.

82 A multiple chromosome of *Mermiria* in prophase, the tetrad portion granular, the accessory chromosome homogeneous.

83 An oblique view of a first spermatocyte metaphase complex of *Hesperotettix pratensis* showing the eleven separate chromosomes with the accessory chromosome attached to one of the tetrads.

84 Multiple chromosomes in prophase, at *a* and *b* of *Hesperotettix speciosus*, at *c* of *Mermiria*. The accessory chromosome, X, is homogeneous and does not show its longitudinal cleft, while the tetrads are granular and cleft.

85 Polar view of second spermatocyte metaphase of *Boopedon nubilum*, including accessory chromosome.

86 Polar view of first spermatocyte metaphase of *Boopedon*, complete complex.

87 Lateral view of first spermatocyte metaphase spindle of *Hesperotettix pratensis*, the multiple chromosome in black.

88 to 91 Successive stages in the concentration and division of an unequal tetrad of *Phrynotettix magnus*. The shorter member, at the left in each figure, remains granular until complete separation in the metaphase. It is apparent that this is an equational division of each univalent part of the tetrad (see figs. 125 and 126 for photomicrographs of chromosomes drawn in fig. 88).

92 and 93 Polar views of first spermatocyte metaphase of *Mestobregma plattei*, showing variation in number of rings.

94 to 98 From *Chortophaga viridifasciata*.

94 and 98 Polar views of spermatogonial metaphases; in the former there are four tetrads, in the latter all the elements are separate.

95 Polar view of second spermatocyte metaphase of *Chortophaga* containing the accessory chromosome.

96 and 97 Lateral views of first spermatocyte metaphase complexes arranged in order of the sizes of their members. The variation in the form of the chromosomes with the stage of division shown. Chromosome 12 of figure 97 is a lateral view of a ring in which the contrasynaptic ends are extended like the synaptic.

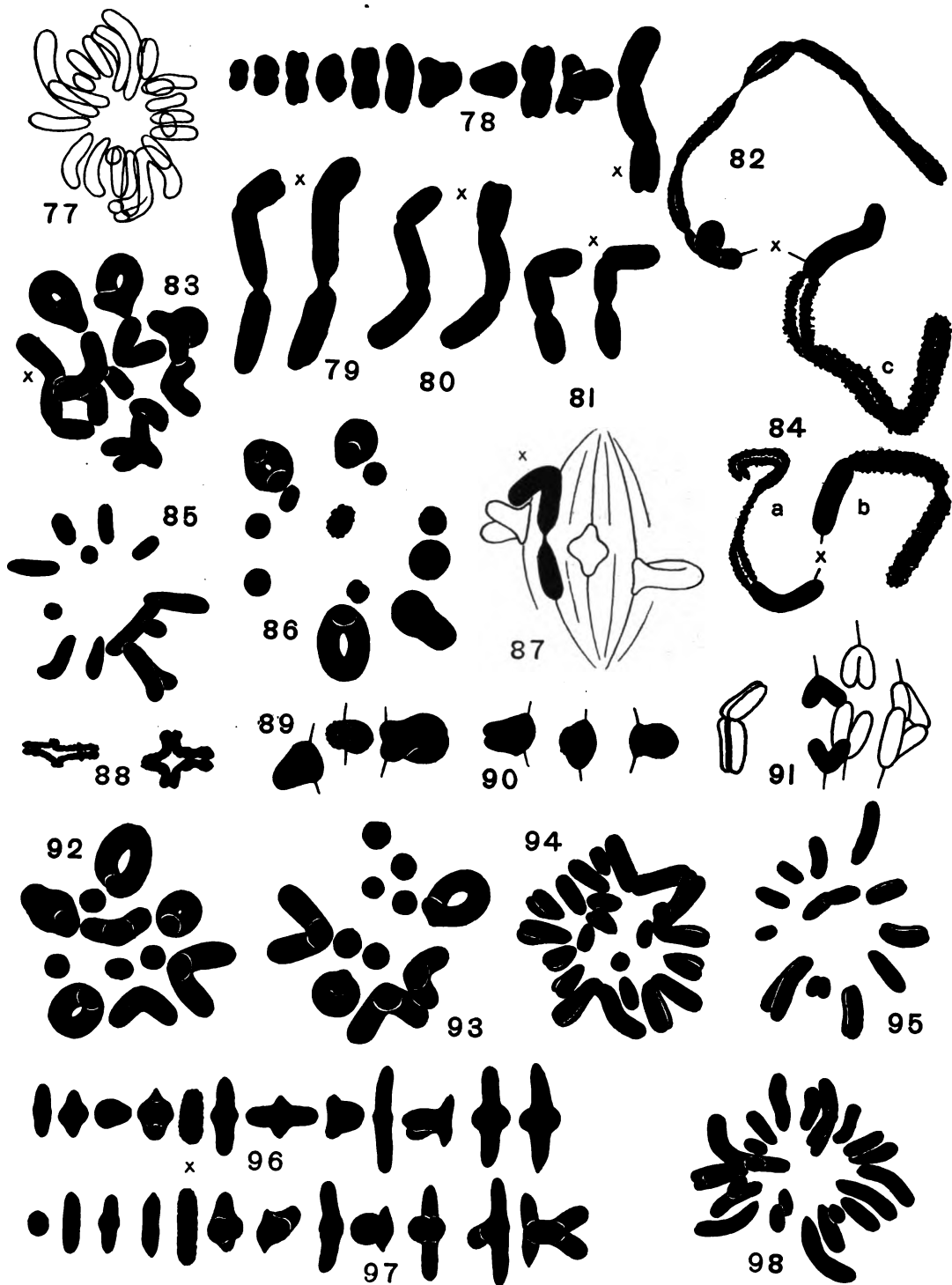


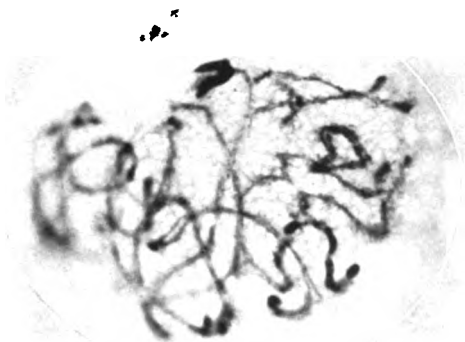
PLATE 7

EXPLANATION OF FIGURES

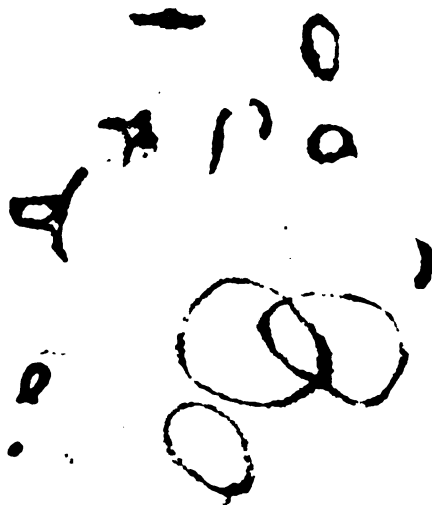
99 Photograph of a smear of first spermatocyte prophase of *Hippiscus*, showing the early longitudinal division of the tetrads, the early concentration of certain tetrads, and the homogeneous appearance of the accessory chromosome. $\times 875$.

100 Photomicrograph of a smear of a first spermatocyte prophase complex of *Trimerotropis maritima*. One of the twelve chromosomes, near the metaphase chromosomes, is very dim, due to spreading. Of particular interest are the four chromosomes on the left. The upper one is a typical rod; the second, a cross; the third, a ring. In each of these the structure is very clear. Just below the prophase ring is another from a metaphase complex. That the latter represents merely a more condensed condition of the former is very evident. The three lower rings are much extended by smearing, but still show their composition. There is no indication of crossed-over ends—they are true rings. $\times 875$.

101 Photomicrograph of a smear of a *Hippiscus* first spermatocyte prophase. The longitudinal division in the tetrads is very clear. $\times 875$.



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PLATE 8

EXPLANATION OF FIGURES

102 to 104 Successively later stages in the formation of the prophase chromosomes of *Brachystola magna*. The gradual opening out of the rings is here shown. Smear preparations. $\times 875$.

105 Prophase of *Mermiria*, with a ring near the center of the figure. $\times 875$.

106 and 107 Two views of the same first spermatocyte metaphase of *Mecostethus lineatus*. In figure 106 a chromosome elongated in the spindle axis is shown, in figure 107 another chromosome lying in the equatorial plate, with the synaptic ends extended; section. $\times 875$.

108 Late prophase of *Trimerotropis maritima*. The lower chromosome is a double ring showing the divergence of the halves of the primary ring (at the left) to form the secondary ring; smear. $\times 875$.

109 Polar view of first spermatocyte metaphase of *Mecostethus* showing the uniformity of chromosome form; this cell is also drawn in figure 34; section. $\times 875$.

110 View of similar stage in *Tropidolophus*; see also figure 29 for a drawing of this cell; section. $\times 875$.

111 to 113 Metaphase complexes of *Hippiscus* from smears. In this species one ring is always provided with the elongations terminating in knobs, from the synaptic ends, to which the fibers attach. $\times 875$.

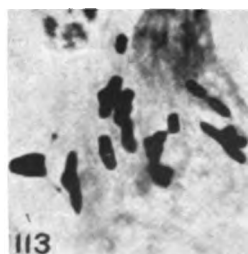
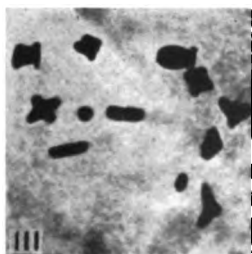
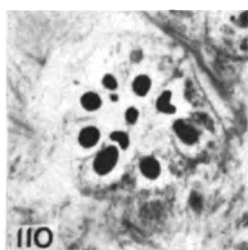
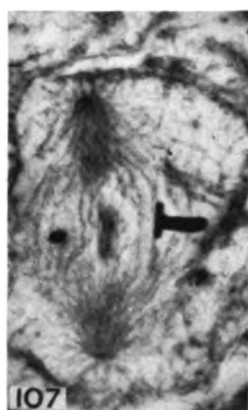
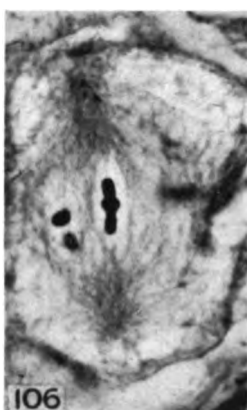
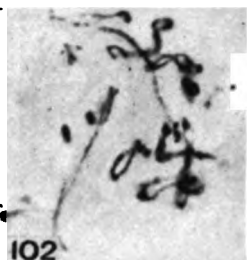


PLATE 9

EXPLANATION OF FIGURES

114 to 124 First spermatocyte prophase from *Mermiria*; 114 to 113, $\times 525$, from sections; 119 to 124, $\times 875$, from sections, except figures 122, 123.

114 Fragment of ring with the opening at the synaptic point.

115 A complete ring with the synaptic ends well drawn out and showing the extension of the ring cleft into these arms.

116 Another view of a ring chromosome.

117 A rod-shaped chromosome.

118 Oblique view of ring.

119 Oblique view of ring showing split extension.

120 View of a cross, *en face*.

121 Lateral view of a ring showing its division into super-imposed rings.

122 Rings and double rings; the lower chromosome presents clearly the structure of the double rings and shows that they are not merely looped structures.

123 Various rings in late prophase.

124 Lateral view of ring.

125 to 128 Prophase chromosomes of *Phrynotettix magnus*; sections. $\times 875$.

125 and 126 Show the unequal tetrad *U* at successive stages of development (see fig. 88).

127 Oblique view of a ring.

128 Bent rod.

129 to 133 Views of first spermatocyte metaphase complexes of *Mermiria*; sections. $\times 525$.

129 The upper cell shows a polar view of a complex with perichromosomal clear spaces, and with the centrally placed synaptic ends of the ring chromosomes dark.

130 Upper cell as in figure 129; in the lower cell a lateral view of one ring.

131 In the upper cell a rod shaped chromosome, *en face*, with central opening indicated.

132 Polar view of first spermatocyte metaphase of *Mermiria* showing clearly the rings and V's lying in the plane of the equatorial plate.

133 Oblique view of a ring in the upper cell demonstrating the symmetrical position of the synaptic extension with reference to the two sides of the ring (cf. fig. 127).

134 to 138 From the *Stenobothrus*-like form; sections. $\times 875$.

134 Polar view of spermatogonial metaphase showing chromosomes with terminal and subterminal fiber attachments.

135 Lateral view of first spermatocyte metaphase in which a ring composed of two spermatogonial chromosomes with subterminal attachments is separating into these two elements. The longitudinal cleft is apparent in the disjoined ends.

136 Lateral view, as in figure 135, showing other forms of chromosomes.

137 Polar view of second spermatocyte metaphase showing chromosomes with terminal and subterminal attachments.

138 Upper cell as in figure 137; lower cell, lateral view.

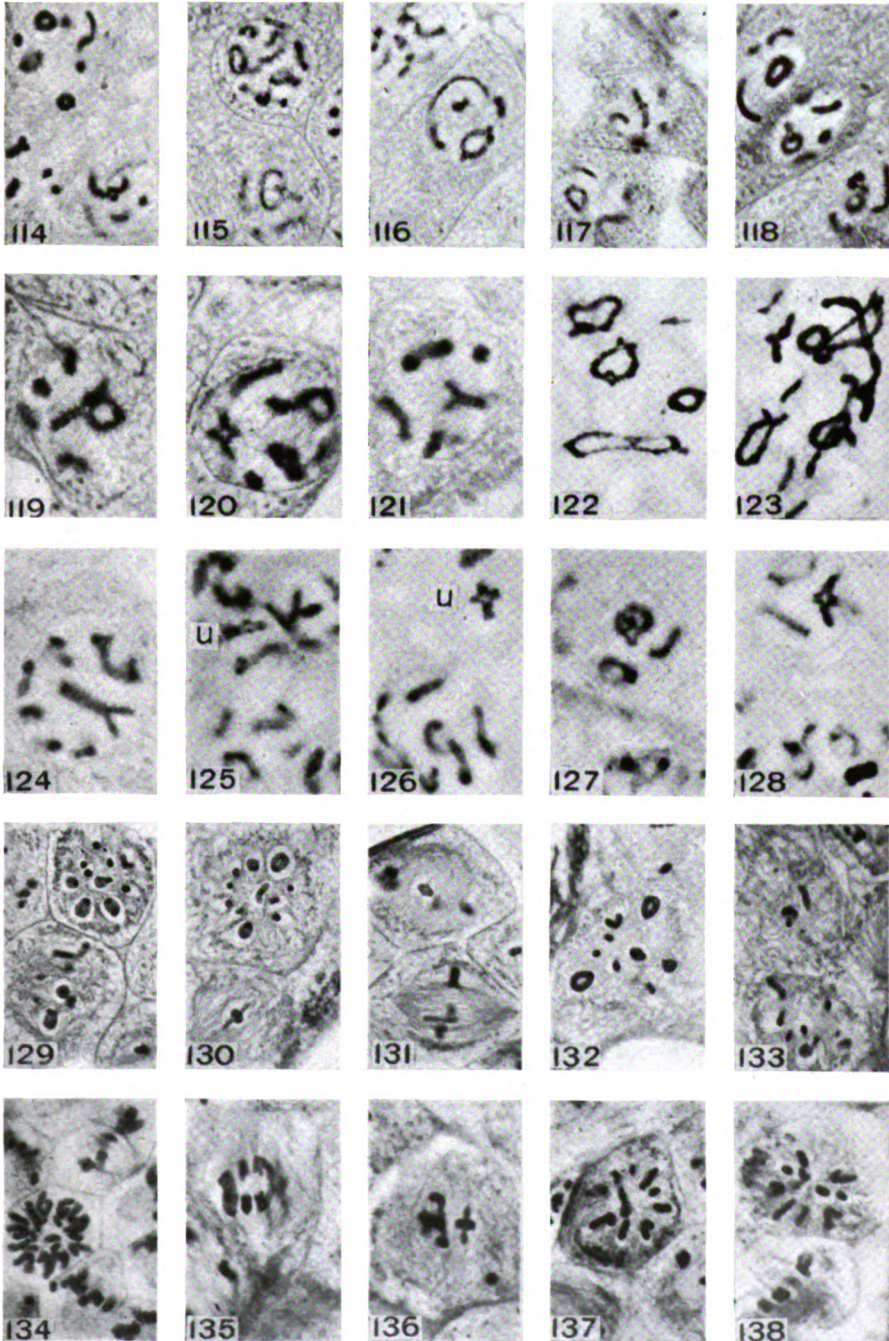


PLATE 10

EXPLANATION OF FIGURES

139 Copy of Davis' text figures *Oa, Ob, Oc*, rope models to explain his conception of the structure and method of division of the ring chromosomes. From these it will be observed that the rings lie in the plane of the spindle axis and that the longitudinal cleft is, therefore, not in the equatorial plate.

140 Photograph of a series of wax models of the chromosome complex of *Tropidolophus* shown in figures 29 and 110. These were prepared after a careful study of the group and they were arranged in the same relative positions in which they appeared in the cell. The central opening of the rings was purposely made larger in order to exhibit their structure more clearly. Compare these with figure 139 in order to see most clearly the difference between the conception of ring formation advocated by de Sinéty, Davis and Montgomery and that held by myself and students for the *Hippiscus* type.

141 Copy of Granata's text figure 1 illustrating his interpretation of tetrad formation in *Pamphagus*. His view of the formed tetrad is essentially similar to my own, even to the compound ring.

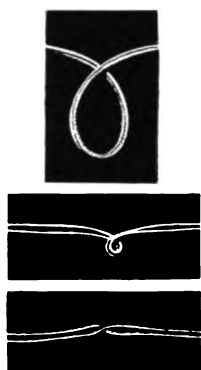
142 Copy of Morse's text figure 1, showing various interpretations. The series in the first row are copies of Sutton's figures and represent my own conception. The second row presents Davis' view, the third is Morse's explanation of the chromosome forms in *Periplaneta*, and the fourth is to illustrate the Schreiner's interpretation of *Tomopteris*. This series of figures represents clearly the various interpretations which I have discussed elsewhere.

143 Photograph of the ring- and cross-shaped wax models shown in figure 140. These demonstrate that such forms are indistinguishable from each other when viewed *en face*, and also that rings constituted as I have described them, when viewed obliquely, appear as loops with crossed ends.

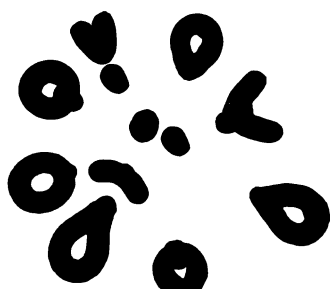
144 Diagrams by Otte to illustrate his conception of a double cross division, with chromosomes of similar appearance in first spermatocyte (E, F) and second spermatocyte (H, J). Compare these with the figures of these generations of cells in various *Orthoptera* given in this paper.

145 Figures 148 and 149 of de Sinéty to show the method of division in chromosomes with terminal (A), subterminal (B), and median (C) fiber attachment. Figures marked A illustrate the conception of ring division first advocated by this author and later supported by Davis (fig. 139) and Brunelli (figs. 146-147). Those marked A' are to explain chromosomes of the first spermatocyte metaphase in lateral view with diverging ends where the fibers attach and at the opposite side. These are in reality rings with the contrasynaptic ends extended.

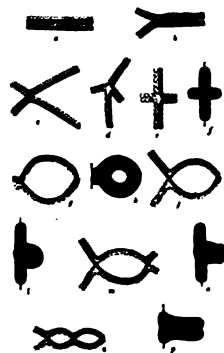
146 and 147 Copies of Brunelli's figures 11, 12, 14, 15, illustrating his ideas of chromosome division in the first spermatocyte. These are essentially like those of de Sinéty.



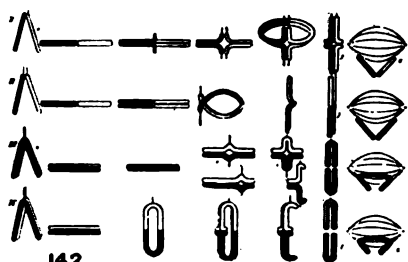
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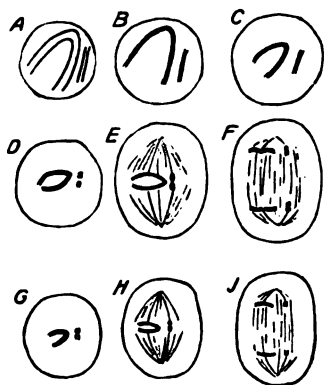
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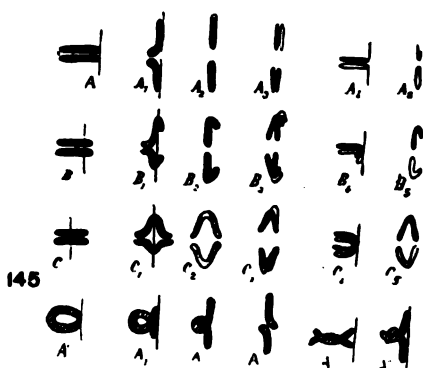
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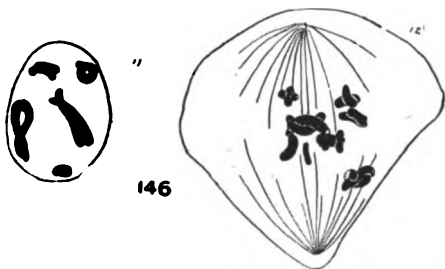
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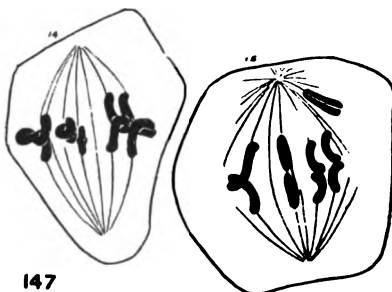
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Thesis presented to the Faculty of the Graduate School of the University of Pennsylvania in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

THE SPERMATOGENESIS OF ASCARIS MEGALOCEPHALA WITH SPECIAL REFERENCE TO THE TWO CYTOPLASMIC INCLUSIONS, THE REFRACTIVE BODY AND THE 'MITOCHONDRIA': THEIR ORIGIN, NATURE AND RÔLE IN FERTILIZATION

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FORTY-EIGHT FIGURES

In 1906 Marcus made two notable contributions to our knowledge of nematode spermatogenesis. The first was his discovery that the refractive body is formed within the vas deferens in *Ascaris canis*, thus completing the spermatogenesis within the male. Previously this most unique and characteristic structure of the nematode spermatozoon had been seen only in those cells which had already entered the uterus of the female. This discovery was confirmed by Mayer ('08) and Romieu ('11), both working with *A. megalocephala*.

Marcus also suggested that the 'protoplasmic corpuscles' or 'microsomes,' observed by Van Beneden, Hertwig and others surrounding the chromatic mass in the spermatid, were mitochondria. This interpretation has been supported by Mayer ('08), Romieu ('11), Meves ('11) and Faure-Fremiet ('11), all working with *A. megalocephala*.¹

But none of these workers made any attempt to learn the origin or to trace the development of either the refractive body or the mitochondria up to their appearance in the complete spermatozoon. It seemed very desirable to do this, now that we know that the entire development of the paternal germ cell takes place

¹ Since this paper was written, Romeis has published a study of the degeneration of the 'condriosomes' in *Ascaris spermatozoa*, in which he also calls these bodies mitochondria.

within the male. At the suggestion of Prof. T. H. Montgomery, Jr., I undertook this study. I wish here to express my deep appreciation of Professor Montgomery's kindly interest in my work, and of his helpful advice and criticism, during the course of the observations. I very much regret that this report was not finished in time to be reviewed by him, for this is a field of research in which Professor Montgomery stood preëminent.

THE REFRACTIVE BODY

Place and manner of its formation

Notwithstanding the fact that the spermatogenesis of the nematodes has been studied since 1858, the history of the refractive body had remained unknown. During this time, the study of no animal has contributed more to our knowledge of the cytology of the germ cells than that of the common nematode parasite of the horse, *A. megalocephala*. The brilliant researches of Van Beneden, Hertwig, Brauer, Tretjakoff and others leave little to be desired concerning the nuclear phenomena during spermatogenesis. The laws of nuclear structure and behavior here discovered have been found to be of very general application. But the observations of these authors on cytoplasmic structures, accurate and extensive as they are, lack the completeness of a final interpretation, because none of them saw the last stage in the process of sperm development.

Throughout the incomparable researches of Van Beneden on the development of the *Ascaris* spermatozoon there is no indication that he even suspected the formation of the refractive body within the vas deferens. His excellent figures represent the various stages of its development as entirely within the uterus. He believed that the paternal element left the male in the form of the spermatid, spherical or slightly conical in form but with no trace of the refractive body.

At the proximal end of the uterus one always finds a great number of spermatozoa crowded among the eggs and uterine epithelial cells. This region Van Beneden named the 'receptaculum seminis.' Among this crowd of spermatozoa are to be

found some that are only slightly conical, with no trace of any inclusion; some having a thin axial rod in the cone which stains readily in iron hematoxylin; some in which this rod is thicker and longer, and more deeply staining, and others contain the fully formed refractive body. In these four forms or 'types' Van Beneden thought he saw the complete development of this body. His reasoning seemed so logical and his figures so in keeping with all observations that most of the students in this field have accepted his conclusions.

O. Hertwig ('90) and Brauer ('93) did not follow the development of the sperm beyond the formation of the spermatid. These authors gave special attention to the nuclear phenomena, although they describe and figure cytoplasmic inclusions as they appear throughout the growth period and maturation divisions.

Tretjakoff ('05) followed Van Beneden in a study of the complete spermatogenesis, and concluded with him that the spermatid enters the uterus, there to become the spermatozoon. He points out that in many animals the uterine epithelial cells act as nurse cells for the developing spermatozoa, and he interprets the crowding of the spaces between the epithelial cells of the uterus wall with sperm cells of the four types of Van Beneden as an effort to get at this necessary food supply.

But Marcus showed that, in *A. canis*, throughout the later growth period, maturation divisions and also in the spermatid, the cytoplasm contains numerous bodies, rodlike or spherical in form, which in the living cell are refringent, like the refractive body, and in fixed and stained material resemble that body in all staining reactions. In his study of the developing spermatid Marcus finds that these bodies, now uniform in size and spherical in outline, gradually fuse together and thus ultimately form the refractive body itself. Mayer showed that this body is formed in exactly the same way in *A. megalocephala*. His work is confirmed by Romieu,² and I have seen the same phenomenon.

² The authors just named explain the long delay in the discovery of the place and manner of formation of this body. They find that in only one male out of thirty killed is the development of the sperm cells in just the right stage to show this formation. This proportion is so small that we are not surprised that the

Thus the destiny of the refringent granules and vesicles, which have long been observed in the spermatocytes and spermatids of nematodes and of *A. megalcephala* in particular, is no longer a mystery. Every observer since the time of Munk ('58) has seen them, and many have been struck with the similarity between the appearance of them and the refractive body, both in life and in fixed and stained material. Indeed, Nussbaum ('84) and Schneider ('02) suggested their true relationship, but neither saw any proof of it. Now, however, we have this proof, and the search for the true origin of the refractive body leads us to a study of the complete history of the refringent vesicles.

THE REFRINGENT GRANULES AND VESICLES

Technique

These bodies are readily seen in unstained, living cells as spherical, shining granules or droplets scattered throughout the cytoplasm. No division has ever been observed in them, though if it occurred it could easily be seen. Such intra vitam stains as neutral red, methyl-green or methyl-blue are readily taken by them. But some of the nuclear structures also take these stains. They are fixed equally well by all the osmic fixatives and by most of the acetic mixtures. After fixation, as well as in life, the similarity of their staining reactions to those of certain nuclear structures is striking. Most of the stains in common use give results which would lead one to conclude that these refringent bodies are made of nuclear material escaped into the cytoplasm. Indeed Scheben ('05), Struckman, and Auerbach all agree with Munk ('58) in the belief that the refractive body exudes from the nucleus, reasoning only from the similar staining reactions of this body and the chromatin.

Although it is upon such evidence that we must rely for our knowledge of the true relationship of the various structures in the cell, both nuclear and cytoplasmic, the accuracy of our conclusions will depend entirely upon the selectiveness of the stain

earlier workers considered the absence of the mature spermatozoon in the male to be the normal condition; and even if the rare case of its presence was observed it was probably interpreted as abnormal or the result of poor technique.

we use. For example, osmic acid stains all cytoplasmic inclusions in the developing sperm cell black or gray-black, while iron hematoxylin stains them all dark blue or black. But it would be quite inaccurate to say on the evidence given by these stains that all these bodies are composed of fat, or that they are all made of chromatin. For if we use a counter stain with the iron hematoxylin, such as Bordeaux red, we find that certain of these bodies retain the blue-black color while others readily give it up and take the red. They must therefore be quite different in chemical nature.

The physiological cytologist insists that the true chemical nature of no protoplasmic structure can be told by its reaction to a stain. Many workers in this field have shown that the staining reactions of such material are almost wholly determined by the ionic sign of the fixative used, and so give little or no information concerning the chemical nature of parts. This is true; yet when two structures always stain differently after a given fixative it is fair to conclude that they are unlike chemically. But the converse of this statement is true only when we use a very selective stain. In the hope of finding a truly differential stain, one which would show in this broad way a difference of chemical nature, I used in the present study combinations of the following fixatives and stains: Flemming's strong solution, without stain; Benda's modification of this solution and his 'krystal violet' stain; Altmann's fixative and his acid fuchsin stain, washed in picric alcohol. Besides these reciprocal stains suggested by the men who designed the fixatives, I used the Ehrlich-Biondi-Heidenhain preparation and iron hematoxylin-Bordeaux red as double stains, not only after these fixatives but also after Carnoy and Lebrun's fluid, sublimate-acetic, zur Strassen's, Tellysnickski's, Zenker's, Müller's, and Bouin's fixatives. The unstained osmic preparations are of little value from the point of view of differentiation, no matter how carefully treated. Altmann's acid-fuchsin-picric-alcohol method is somewhat better, but it does not differentiate clearly the refractive body and the mitochondria or 'plastochondria' (Meves) from chromatic structures. But most of the acetic fixatives show good differentiation

of both nuclear and cytoplasmic structures when followed by either iron hematoxylin-Bordeaux red, or the Ehrlich-Biondi stain. These combinations and those suggested by Benda were indeed the only ones that proved to be of value in this study.

The most instructive reactions obtained were those caused by Benda's fixative and stain.³ A late spermatocyte with this technique shows the numerous refringent bodies stained blue, the karyosome or chromatic nucleolus red-brown, and the plastosome and certain small granules throughout the cytoplasm (Meves' 'plastochondria') yellow. While these different colors tell us nothing concerning the chemical composition of these structures we must conclude that they are not alike chemically. The prime value of Benda's stain for our present purpose, therefore, is that it is more selective than any other used. The other combinations mentioned will distinguish the plastosome and small granules from the karyosome and the refringent vesicles, but the latter so closely resemble each other in color that they might easily be

³ Owing to frequent injury to sections in following Benda's directions for staining, I modified his method slightly. As used by Benda, Meves, Duesberg and others, the treatment of material for this stain is as follows: after fixation in strong Flemming's or Hermann's fluid, the tissue is washed in water, then left in a mixture of equal parts of pyroligneous acid and 1 per cent chromic acid to mordant. Then for a like period of time, say twenty-four hours, in a 2 per cent solution of bichromate of potash for the 'second chromization.' The material is then washed, dehydrated and imbedded as usual. After sectioning, iron alum in a 2 or 4 per cent solution is used as a mordant, and also an aqueous solution of sodium sulfalizarine. After washing in water, a solution of 'krystal violet' is poured over the sections and the slide is held over a flame until steam arises from the solution. The stain is then poured off and the sections are washed in water. Next they are destained in 30 per cent acetic acid, and after being thoroughly rinsed in water they are dried between sheets of blotting paper. The sections are then put directly into absolute alcohol for a moment, and are cleared and mounted as usual. I found, however, that heating the sections, destaining them in 30 per cent acetic and finally drying them between blotting or filter paper ruined most of the sections. I got just as clear-cut and permanent stain differentiation by using the following method: after washing the excess alizarine solution from the sections by carefully dipping the slide into water, they were stained by putting the slide into a 3 per cent solution of 'krystal violet' (3 cc. aniline stain in 100 cc. distilled water) for ten minutes. They were then rinsed in water and put into 80 per cent alcohol for about five seconds; then into 95 per cent and finally into absolute alcohol, where the destaining was watched carefully. Then the sections were cleared and mounted as usual.

considered to be alike chemically. But with Benda's stain such a misinterpretation could not be made; for the red-brown of the karyosome and the karyochromatin is quite distinct from the deep blue of the refringent vesicle.

What then does the use of Benda's stain teach us concerning the material which makes up these vesicles before they appear in the late spermatocyte?

Origin

There is no trace of this blue-staining material in the cytoplasm of the spermatogonium, neither during division stages nor in the 'rest stage' nor yet in the synizesis period. But within the nuclear membrane throughout all of these stages a blue-staining material is unmistakably present. It is most abundant in the prophase of the spermatogonial divisions, where it lines the nuclear membrane in thin sheets and irregular masses, apparently covering the karyochromatin (figs. 2 and 3). Often however the karyosome itself is cut, showing a red-brown color inside, though it is covered by the blue staining material. During division this material is seen covering the chromosomes. Figure 1 represents a fortunate section of a cell in metaphase in which two chromosomes were cut nearly lengthwise. The karyochromatin at the center of each is stained red-brown while the surfaces are blue.

Throughout the synizesis period the chromatic skein is in contact with the nuclear membrane at many points. As this period closes, the greater part of the blue-staining material passes toward the nuclear wall, while the karyochromatin and the plastochromatin aggregate to form the karyosome and the plastosome respectively. As a rule these bodies take positions near the center of the nucleus.

The young spermatocyte thus shows a fairly definite localization of three nuclear materials, which stain quite differently from each other (fig. 4). The migration of the blue staining material does not cease when it reaches the nuclear membrane, though as a rule practically all of it has come to line the nuclear wall in the form of cords and plates before any of it pushes through the membrane into the cytoplasm (fig. 4). In figures 4 and 5

we see the actual penetration of the nuclear membrane by this material.⁴ Once in the cytoplasm it takes spherical shape as tiny granules, or as droplets easily visible under the low power lens, according as the quantity of material transuding at that point is larger or 'smaller. These droplets have long been known as the 'refringent granules.' Their refringent appearance, however, is acquired after they have reached the cytoplasm. It is due to the formation of yolk within them, as will be shown later. The material of which they consist is therefore a yolk-forming substance.

Such cytoplasmic inclusions in gland and sex cells, controlling secretion there and staining blue after Benda's stain, are called by many cytologists 'mitochondria.' But these are thought to be wholly cytoplasmic in origin, having nothing to do with the nucleus or its contents. We cannot therefore use this term to represent these yolk-forming granules in *Ascaris* spermatocytes, for they arise in the basichromatin or karyochromatin of the nucleus and pass out into the cytoplasm.

Meves uses the term 'plastochondria' to represent cytoplasmic granules arising in the oxychromatin or plastochromatin, and so indicates their origin and nature. I would suggest the term 'karyochondria' for these yolk-forming granules, because it likewise indicates their origin and nature.⁵ The observation of the migration of the karyochondria from the nucleus leads to an

⁴ I am indebted to Professor McClung for confirming this observation.

⁵ The following table will define the terms 'karyochondria' and 'plastochondria' as they are used in this paper:

	<i>Origin</i>	<i>Function</i>	<i>Equivalents</i>
Karyochondria	Arise in the basichromatin: i.e., chromatin network, chromatin nucleolus or karyosome, and chromosomes	Yolk-forming granules, which very soon become yolk vesicles. These fuse to form the 'refractive body' of the spermatozoon	Cytoplasmic nucleoles Pseudochromosomes Accessory nuclei Vitelline body Trophochromatin Ergastoplasm of Prennant and Bouin Mitochondria of Meves and Korff

interesting interpretation of the condition of the cytoplasm of the spermatocyte as shown in figure 10. Here we find granules and yolk vesicles of varying sizes scattered throughout the cytoplasm. Small granules may be close to the cell wall, while near the nucleus we may find fully formed yolk vesicles. Since the karyochondria do not arise *de novo* in the cytoplasm there must be a constant movement of the cytoplasm, or these inclusions could not become thus scattered. Such protoplasmic movement is well known in resting plant cells, and it occurs, of course, in all dividing cells; but it must occur also in the resting spermatocyte of *Ascaris* (figs. 6 to 10).

Development

The karyochondria, almost as soon as they first appear in the cytoplasm, increase in size and become less deeply staining. They remain spherical in outline as they grow; no irregular or dumb-bell shapes appear amongst them. Though their growth seems to be quite definitely limited, ceasing when the diameter is about ten times that of the original granule, I have not been able to find undoubted cases of division or even fragmentation. For some time before the maturation divisions, the cytoplasm is about half filled with vesicles evenly distributed through it and uniform in size and staining reaction. A count of the vesicles in the median plane of such a cell corresponds so closely with one made in a cell in the midst of the growth period, when only a few of the granules have become fully formed vesicles, that it

Plastochondria	{	Arise in the oxy-	Minute granules	Mitochondria of
		chromatin: i.e.,	which make up	Marcus, Mayer,
		the linin net-	the intervalveolar	Romieu, etc.
		work, true nu-	reticulum, the	Microsomes
		cleolus or	fibrillae, the rays	Plastidules
		plasmosome or	and fibers of the	Archoplasm
		plastosome, the	division figure.	Protoplasmic cor-
		centrosome and	After reaching	puscles.
		centrosphere	the egg cyto-	Paraplasm or met-
			plasm they dis-	aplasm (Wilson)
			solve and help to	
			form the fertili-	
			zation membrane	

is evident that no division occurs among the vesicles. Their number, which is remarkably uniform in all the cells of a tube, seems to be determined, therefore, by the amount of transuding karyochondria.

Destiny

Just before the first maturation division the centrosomes emerge from the nucleus. The refringent vesicles at once begin to arrange themselves in concentric arcs about them. This symmetrical arrangement is kept throughout the divisions. The close of the anaphase of the second division is marked by two interesting events; the fusion of the centrosome with the two chromosomes in each spermatid, and the withdrawal of small, dense granules (plastrochondria) from the refringent vesicles (fig. 34).

When the new spermatids separate they are spherical, and in them the vesicles are arranged radially and concentrically in four or five layers. Each spermatid has at its center the fused centrosome and chromosomes, surrounded by a clear mass of cytoplasm, or the 'perinuclear zone' of Van Beneden. In this zone lie the plastrochondria, or 'microsomes' of Van Beneden. In the great majority of males killed one finds only spermatids of this form filling the vas deferens (figs. 12 and 35). Among them, however, are many which show 'cytoplasmic reduction,' a phenomenon now recognized in the development of the spermatids of a good many animals. In these, probably the older ones, a mass of cytoplasm containing plastrochondria but without refringent vesicles, gradually flows out from the spherical spermatid and is cut off from it (figs. 13 and 14). Romieu ('11) was the first to describe this phenomenon in *Ascaris*. As a result of this loss, the volume of the spermatid is often reduced one-half, and the vesicles are thus brought close together.

In extremely rare cases a male will be found in which the spermatids are transforming into spermatozoa. This transformation probably occupies only a few minutes, and takes place just before copulation occurs.

In these the vesicles gradually fuse together until they form three or four large globules which surround the clear zone (figs. 25 and 36). Indeed, this is usually encroached upon, and some-

times the central mass is temporarily pushed aside (fig. 37). Fusion continues, however, until a single hemispherical body is formed which comes to lie in the cytoplasmic cone of the spermatid, stretching the latter until it forms a mere sheath around it (figs. 15 and 38). The refractive mass now elongates, taking the form of a truncated cone. Its base is slightly concave, and adjoins the clear zone, which has regained its former position (fig. 48).

The refractive body is now completely formed. Before the fusion of the refringent vesicles begins sections of them show a different reaction to stains on the surface and in the interior. This difference is very marked in sections of the globules and of the refractive body itself. While the surfaces of all these inclusions remain a deep blue after Benda's stain the interiors of them become more and more yellowish as fusion progresses, and finally the entire inner portion of the refractive body stains yellow. In the older spermatozoa even the surface of this body loses the blue color (figs. 12, 15, 16 and 25).⁶

I believe this is the final step in the history of the blue-staining material, or karyochondria, in *Ascaris*; that it is entirely consumed in the formation of the refractive body of the spermatozoon by changing chemically into the material of which that body is made, namely, yolk.

For we know that all secretions whether glandular or yolk, stain yellow after Benda's stain. Indeed, Bouin ('05) considers the growing spermatocyte homologous with the gland cell so far as the secretion of yolk in it is concerned. We know that in many gland cells a substance appears in the cytoplasm just before secretion begins, and gradually disappears as the secreted material accumulates (Faure-Fremiet, '10, for literature). This substance has been called a 'prezymogen'—a factor which directs secretion, and may later transform into the secretion itself. In other forms this factor is known as 'mitochondria,' and its behavior seems to be followed precisely, as we have seen, by the karyochondria in *Ascaris*. Mitochondria, however, are considered to be wholly cytoplasmic in origin.

⁶ Romeis ('12) confirms this observation.

RÔLE OF THE REFRACTIVE BODY

In our search for the nature and function of this unique structure, a review of the normal behavior of the *Ascaris* spermatozoon will be of interest. So far as we know at present, these cells are injected into the distal end of the uterus at the time of copulation, and have to make their way to the proximal region of this organ by their own powers of locomotion, for the unfertilized eggs are to be found only at the proximal end. The uterus is usually not less than 12 and may be more than 15 cm. in length, and it is always full of eggs and developing larvae. The spermatozoa have, therefore, a relatively long and difficult journey to accomplish; and since their only known method of locomotion is amoeboid creeping, their rate of travel must be very slow. Indeed, the total distance to be traversed by them is far greater than the length of the uterus, because the inner walls of this organ are thickly studded with epithelial papillae, so that their course cannot be a direct one. It is evident, therefore, that a considerable amount of energy must be expended by the spermatozoa in making this journey, and hence a definite food supply is necessary for their use.

A very brief examination of the sperm which have reached the proximal or 'entrance region' of the uterus shows a great variation in the form and size of the refractive body, as we have seen. While Van Beneden interpreted these various shapes as indicating different stages in the growth of this structure, basing his opinion upon the hypothesis of the uterine origin of it, Mayer, Romieu, Romeis and others interpret them as stages in the phagocytosis or degeneration of superfluous spermatozoa. I believe, however, that they merely mark the normal consumption of a proper food supply by the spermatozoon itself. While figures 40 and 48 represent sperm in which very little change in the form of this body has taken place, such are rare. In figures 40, 46 and 47 are shown those in which only a remnant is left in the form of a corroded axial rod.⁷ In figures 39, and 41 to 45

⁷ Since iron hematoxylin used after any acetic fixative stains both the inner and outer parts of the refractive body blue-black, it is an excellent stain to use to reveal even slight traces of this structure.

spermatozoa are represented which not only have lost all trace of the refractive body, but are entering eggs in this condition. In sections of one tube I found that nearly half the eggs had been entered by spermatozoa of this kind. To be sure that these were not merely lying upon the sections, I examined many whole eggs which had been fixed and stained just as the sectioned eggs were, and found many eggs which were being entered by such spermatozoa. I have examined a great many egg tubes and find them all alike with respect to this phenomenon (figs. 42 to 45).

Probably no point in the study of *Ascaris* spermatogenesis has called forth greater difference of opinion than the nature and function of the refractive body. Van Beneden first observed spermatozoa entering eggs without it, and concluded from this fact that it plays no part whatever in fertilization. His further conclusion that its presence in the spermatozoon is purely accidental must be judged in the light of the fact that he had seen this structure only in the proximal region of the uterus, and thought it was formed there.

Boveri agrees with Van Beneden that it is not necessary for fertilization, since this is accomplished with or without it. Scheben, on the other hand, believes it to be most important in fertilization, for he thinks it gives rise to the male pronucleus. Tretjakoff believes that its only function is to serve as a mechanical support for the sperm, corresponding in this respect to the capsule of the decapod spermatozoon as interpreted by Koltzoff.

Romieu agrees to this suggestion, but adds that it may feed the egg as well as to help in effecting penetration. Romieu thinks that its presence is the sign of maturity of the sperm, and that it is present in every functional male cell.

Marcus suggests that in *Ascaris canis* it may be a food supply. On page 460 we read, "Ich muss mich also in Gegensatz zu Scheben der Ansicht v. Benedens und Boveris ausschliessen die dem Glanskörper keine unmittelbare Bedeutung bei der Befruchtung zuschreiben. Nach meiner Auffassung ist er ein Nahrungskörper. Ob die Dotterkugeln bei den Spermatocyten ursprünglich aus dem ins Plasma hinausgetretenen Trophochromatin entstanden sind, kann ich nicht entscheiden." He adds

that this may occur, but he thinks it is not probable because the yolk forms first at the periphery of the cell.

Marcus thus approaches very closely the true origin and nature of the refractive body. He lacked only the actual observation of the escape of the 'trophochromatin' or karyochondria from the nucleus into the cytoplasm, and the formation from this of the refractive vesicles, to complete his history of the refractive body in *Ascaris canis*. As we have seen, this gap is filled by this study of *A. megalocephala*. If it be true that the yolk vesicles first appear near the cell wall in *A. canis*, it must be due to chance aggregation of them there as a result of protoplasmic movement. The smaller granules from which others would be derived must be scattered throughout the cell, though these might have been overlooked.

At any rate, the suggestion of Marcus is the correct one—borne out fully, I believe, by the observations recorded in this paper—that the refractive body plays no essential part in fertilization, but that it is merely a food supply for the spermatozoon alone, derived from the cytoplasm through the activity of a substance which escapes from the nucleus, the karyochondria.

THE 'MITOCHONDRIA'

When spermatocytes are stained in iron hematoxylin alone, after fixation in an acetic mixture, and destained almost completely, one finds small dense granules in the refringent vesicles which stain black. In those cells which are approaching division the vesicles are slightly oval, and the granules arrange themselves in chains or rods which lie in the long axes of the vesicles. They are most conspicuous during the metaphase of the second maturation division, because at this time the refringent vesicles are arranged concentrically around the centrosomes, and the chains of granules are therefore perfectly radial with respect to these (fig. 34).

But during the anaphase of this division the chains of granules are drawn out of the vesicles and break up, and the separate granules crowd close up to the chromatic mass, but in radial and concentric lines. The refringent vesicles, also acted upon by the

attractive force of the centrosome, arrange themselves in concentric circles. The size and number of those vesicles in the nearest circle prevent them from coming up to the attracting mass at the center, so that a clear zone,—the 'perinuclear zone' of Van Beneden, is left around it. In this zone the granules just described stand out clearly.

The vesicles still stain blue in Benda's stain, though of a lighter shade than before the divisions, *but the granules always stain yellow*; and, since they surround the closely massed centrosome and chromosomes, the red-brown of the latter is completely masked by the yellow, unless seen in section (figs. 12). After iron-hematoxylin-Bordeaux red the granules stain red, while the vesicles stain blue; and in Ehrlich-Biondi the granules take the red dye and the vesicles are purple (fig. 25). Thus these granules must be quite unlike the vesicles chemically. Their origin and history will be of interest.

The plastosome and the plastin grains or plastochondria

The plastosome appears in the nucleus of the spermatogonium very soon after division ceases. It is a small granule throughout the 'rest period' and stains yellow after Benda's stain (figs. 3 to 11), bright red after Ehrlich-Biondi (figs. 19 to 24) and black after iron hematoxylin (figs. 29, 30 and 31); while the karyochromatin stains red-brown, green and blue-black respectively, after these stains, as shown in the figures just mentioned.

During the synzesis stage the plastosome increases in size, and often small granules staining just like it are to be seen scattered throughout the nucleus (figs. 19, 20 and 21). Throughout the long growth period these remain evident, and their number increases.

Early in the growth period small granules like those just described in the nucleus appear in the cytoplasm. They are very small, 0.1 to 0.5 μ in diameter (figs. 5, 6 and 7). At the close of the growth period, when the centrosome leaves the nucleus it takes these granules with it into the cytoplasm. They are often clustered around it in the form of a hollow sphere, as shown in section in figure 24. As the cleavage figure is formed, many of

the granules scatter throughout the cytoplasm, and these cannot be distinguished by size, form or staining reaction from those already there.

At about this time one finds similar granules inside the refringent vesicles, as stated above. The plastosome itself sometimes survives the growth period as a unit, though often it does not. It is never found in the spermatid nor the spermatozoon, but in both of these the plastochondria are very conspicuous. Usually it exists only as scattered fragments by the time the maturation divisions occur, and these form the rays and fibers of the cleavage figure. Whether these plastin grains or plastochondria reach the cytoplasm by passing through the nuclear membrane at many points, or by going out with the centrosome they are undoubtedly of plastosomal origin and nature.

But some of these granules develop within the refringent vesicles, escaping from them just after the second maturation division. These cannot have come from the plastosome. Yet their staining reactions indicate that they are exactly like all the other plastochondria. We have, apparently, in this observation, a proof of the true origin of all of the plastochondria, and so of the plastosome itself. Their staining reactions, whether within or outside of the nucleus, show them to be secretion or excretion products. Here we see what substance it is which first secretes and then excretes them. In the cytoplasm the only secreting agent is the karyochondrial material in the form of the refringent granules and vesicles. In the nucleus the plastosome does not appear until this material is present in considerable quantity. I believe we must conclude that the plastosome and its derived plastochondria, therefore—indeed, the plastochromatin in all its various forms—arises in the karyochromatin as a product of its metabolism. Montgomery ('11) reaches this same conclusion, so far as the origin of the plastosome is concerned, from his study of the history of the nucleus of the spermatocyte of *Euchistus*. That the plastochondria cannot be used as food by the spermatozoon is shown by the fact that they are excreted by the refringent vesicles just before these fuse to form the refractive body, which is the food supply of the spermatozoon. Earlier authors

have called them 'plastidules,' 'plastin granules' or 'microsomes.' Van Beneden first observed and figured them clustered around the chromatic mass at the center of the spermatid. But he did not trace their origin. Mayer first saw them in the refringent vesicles, and leaving these to take the position just mentioned. He did not trace their origin, however, and seems to have overlooked the fact that they always stain with acid dyes, for he calls them mitochondria, although all cytologists agree that mitochondria take basic dyes.

When the plastrochondria reach the perinuclear zone fusion occurs to a limited extent, resulting in fewer and larger granules, while many are still scattered throughout the cytoplasm of the spermatid. Many of these are lost when cytoplasmic reduction occurs. Though the diameter of the spermatid is reduced about one-half at this time, the granules lost seem to be only those lying by chance toward the periphery of the cell. There seems to be no selection or segregation here.

Those that remain keep a fairly definitely symmetrical position around the center of the spermatid during its transformation into the spermatozoon, regaining it if it is temporarily lost. The smaller ones lie in the thin sheath of protoplasm which surrounds the refractive body, while the larger ones lie in the sponge-like protoplasm of the 'crown' or head (figs. 25, 27, 28 and 48).

In these positions they are carried into the egg; but, as soon as they enter the egg cytoplasm, the symmetrical arrangement is lost, as a consequence of the absorption of the sperm cytoplasm by that of the egg. The larger ones in the perinuclear zone remain in a fairly close group long after the outlines of the sperm have been lost, and can be distinguished easily by their larger size, as Meves has recently pointed out ('11), from similar granules which are scattered throughout the egg cytoplasm. After the fusion of the pronuclei, however, they also become dispersed, fusing with those of the egg, or with those of their own kind, or remaining as small granules which take part in the formation of the rays and fibers of the cleavage figure. Romeis ('12) confirms the observations of other authors who found that they help to form the fertilization membrane of the egg after they have dissolved in the egg cytoplasm.

This is the complete history I believe, of the 'mitochondria' of Mayer, Romieu, Faure-Fremiet, and Romeis, or the "plastochondria" of Meves. Their behavior is such as any inert excretion grains would and do show in other cells. They undergo no division, show no inherent power of growth, and do not transform into any other substances. They are shifted about in the cell by any forces that are set up in it. Their staining reactions are always those of secretion products, yellow after Benda's stain, and red after Ehrlich-Biondi. They do not take intra vitam stains.

DISCUSSION

Faure-Fremiet's definition of true mitochondria

It remains only to consider briefly the observations recorded in this paper in the light of the study of cytoplasmic inclusions in the germ and body cell of other animals, now recognized as 'microsomes,' 'protoplasmic corpuscle,' 'archoplasm,' 'mitochondria,' 'ergastoplasm,' 'plastochondria,' and so forth, by various authors. The essential point in the study of such inclusions is to determine which of them are living, formative elements, and which are inert, formed products.

Ever since the panstaking work of Dujardin, this problem has claimed the attention of the most eminent cytologists. Faure-Fremiet well says, (page 461, '10):

When we find in a cell a fat globule, a vitelline corpuscle or an albuminoid granule which increases in size until it is absorbed or expelled, we do not consider this an integral part of the cell, since it results from the work of the cell, or it is utilized in this work and does not help to direct it. When we see, on the other hand, the nucleus of the cell or a leucoplast we consider these as part of the organization of the cell, controlling its work. We see them grow and divide like the cell itself, and we know that they are active factors in the life of the cell. We conclude therefore that the nucleus is a *living* factor, while the fat globule is *not*. This illustration is clear cut, but it is often far more difficult to apply the words *formative substance*, and *formed substance*.

His closing sentence on this subject is of interest in connection with this review: "For my part, when I see that these elements divide, that they contain fatty acids, or that yolk material ap-

pears around or within them, I merely say that similar facts have been observed in connection with the leucopasts of plants." These sentences are quoted from the close of the introduction to a very complete review of the literature on mitochondria.

Mitochondria as yolk-forming factors in spermatozoa and in eggs

In this comprehensive study, Faure-Fremiet shows that this material occurs in sex cells under four different types of formation and behavior. In the last of these he includes all those cases in which the mitochondria transform partially or wholly into yolk. The spermatocytes of batrachians and of myriapods, and the oocytes of many groups of animals are cited here.

Prennant ('87), in the spermatocytes of myriapods, observed certain granules and filaments which he named 'ergastoplasm,' because he considered them as active elements in the cell. In 1905 Bouin showed that these bodies transform into yolk. Meves and Korff ('01) call this material 'mitochondria,' as its behavior in the myriapods was like that of mitochondria in other forms.

Benda ('03) found the mitochondria in the spermatocyte of *Rana* in a compact mass in a cavity in the nuclear wall. He called this mass the 'corps condriogens.' Champy ('09) found, here and there, in the spermatocytes of *Bombinator* 'yolk vesicles' of various sizes among the mitochondrial granules and filaments. They resembled the nucleolus inside the nucleus in staining reaction but they are often made of two spheres, the inner one always staining lighter or more acidophilic than the outer. This author believed that yolk is formed in these 'cytoplasmic nucleoles' or mitochondria, just as in the oocytes of *Bombinator* or other forms.

Very clear cases of the transformation of mitochondria into yolk have been found in the developing oocytes of a number of animals. The simplest case recorded by Faure-Fremiet is found in the chilopods, where there is no yolk nucleus or vitelline body of any kind. The mitochondria themselves become yolk granules, then vesicles or globules, just as they do in the spermatocytes of these forms.

Bouin ('98) observed the 'paranuclear bodies' in the oocytes of Echinoderms breaking up into 'corpuscles;' these 'disappeared' at the moment of yolk-formation through loss of their stainability, but the cell seemed to be full of yolk granules and vesicles.

Loyez ('09) found in the young oocytes of certain tunicates the 'nucleoles' grouped around the nucleus. Later they form angular filaments, which then break up into granules. These increase in size, and show a clear, inner portion which does not stain blue in Benda's stain, while the surface does. These vesicles increase in size, the unstained central portion growing at the expense of the blue-staining surface material, until none of it is left. Van der Stricht ('05) finds the 'pseudochromosomes' in the oocyte of the white rat and the bat breaking up into mitochondria, which then transform into yolk. Lams and Doorme ('07) confirm these observations on the oocytes of the rat and the guinea-pig.

Mitochondria in the spermatogenesis of Ascaris megalocephala

Many other cases of this kind might be cited, but these are clear. In view of them my observations of the origin and development of the refractive body in *Ascaris* lead clearly to the conclusion that this form and probably all of the nematodes belong to this fourth 'type' of Faure-Fremiet, for the blue-staining material in *Ascaris* spermatogenesis is certainly a yolk-forming material, and eventually completely transforms into yolk. Its place of origin is also clear. This 'ergastoplasm' of Prenant and Bouin, or 'mitochondria' of Meves and Korff undoubtedly has its counterpart in the blue-staining material in the spermatocyte of *Ascaris*. The authors just named thought that 'mitochondria' were expelled from the nucleus of the myriapod spermatocyte into the cytoplasm. As we have seen, this is the origin of the blue staining material in the *Ascaris* spermatocyte. Here it forms the refringent granule, then the surface of the refringent vesicle and later of the refractive body. It is evident that the term 'mitochondria' is ambiguous, for as we have just seen, it is used to stand for cytoplasmic inclusions which form yolk and

also for those which do not, and which therefore must be quite different in origin and nature. While Meves and Korff use it to represent this yolk-forming material of nuclear origin, Benda, Duesberg and others use it to represent structures which they believe are wholly cytoplasmic in origin, and give rise to the various connective tissues of the embryo. The authors just mentioned state that the mitochondria always take basic stains, though they do not arise in the nucleus. But Mayer, Romieu and other students of *Ascaris* spermatogenesis call the small, dense granules found in the 'perinuclear zone' of the spermatid, and in the 'crown' of the spermatozoon, mitochondria. But these granules not only take acid stains always, but they unquestionably arise in the nucleus. Besides, no one has yet found that they give rise to any yolk or embryonic tissue. They seem to be merely inert residua.

Manifestly, therefore, the term 'mitochondria' is useless. Meves recognizes the inappropriateness of this name for the granules in *Ascaris* just mentioned, and calls them 'plastochondria' because of their resemblance to the plastosome in all staining reactions, and their possible origin in it.

I would suggest the name 'karyochondria' for the yolk-forming granules because of their close relation to the karyochromatin. It remains to be seen, I think, whether all inclusions in the cytoplasm of sex cells of other forms referred to as 'mitochondria' cannot be identified with one or the other of these, the plastochondria or the karyochondria, for there is abundant evidence that both yolk-forming and organ-forming factors first arise in the nucleus and pass out into the cytoplasm, as will be shown later.

Among the earlier students of *Ascaris* spermatogenesis, Van Beneden spoke of Meves' plastochondria merely as 'protoplasmic corpuscles,' attributing no function whatever to them. Hertwig figures them in the refringent vesicles, just before the maturation divisions, but he does not discuss them. Boveri found them in the egg, surrounding the male and female pronuclei. He called them 'archoplasmic grains,' but he attributed no significance to them. Tretjakoff figures them scattered among the

refrigent vesicles early in the growth period, but he does not refer to their origin or behavior.

Meves, however, finds that they come from the plastosome, as we have seen, and he considers them of great importance in fertilization. He interprets the fact that they fuse with similar but smaller granules in the egg as a phenomenon of great significance. Meves was not the first to observe this fusion; as he points out (p. 686), L. and R. Zoja ('91) first described it. But Meves thinks that these observers missed the significance of it. He believes that through the fusion of the plastochondria from the sperm with those of the egg, the male transmits to the offspring all the paternal structural characters that are to be inherited, and that these can be inherited in no other way. Thus, Meves' plastochondria agree in function with the 'mitochondria' of Benda, Duesberg and others; but the former are nuclear in origin, while the latter are thought to arise entirely within the cytoplasm. Even if the male pronucleus could be utterly obliterated before it fused with the female pronucleus, the offspring, according to Meves, would show structural characters inherited from the father.

But we may well ask what proof is there for considering the plastochondria to be of such great biological importance? Meves gives us none whatever except that these granules persist throughout the early cleavages. As we have seen these granules play the part of inert products in the cytoplasm forming the fibers or rays of the cleavage figure, or through their solution helping to form the fertilization membrane (Romeis). We are entirely without evidence of the great importance of these granules in heredity. On the other hand, there seem to be serious reasons for doubting the interpretation of them which Meves has given.

a. Inheritance in enucleated eggs. If Meves' interpretation be correct, Boveri's classic experiment of fertilizing the enucleated eggs of *Sphaerechinus* with sperm of *Echinus* should yield plutei which are not of the pure *Echinus* type, since the egg plastochondria should contribute *Sphaerechinus* structures to the hybrid.

Boveri, however, believes that the plutei so produced are of the pure *Echinus* type. This conclusion shows clearly that the maternal plastochondria are not bearers of structural characteristics, and it cannot be supposed that these bodies in the spermatozoon possess hereditary qualities not to be found in those in the egg.

b. Heredity in hybrids. The experiments of Loeb, Herbst, Baltzer, Tennent and others in crossing echinoderms, fishes, and so forth, show that heterogeneous hybrids are almost constantly maternal in structure. This should never be the case if the plastochondria function in inheritance of structure. But the larvae produced in these experiments show clearly, when studied cytologically, what it is that determines their maternal type of structure. Baltzer, Tennent and others have seen cross-fertilized eggs of echinoderms actually eliminate from themselves certain bodies brought into them by the spermatozoon. But these bodies are not plastochondria; they are chromosomes. Loeb finds that fish hybrids of this kind are always maternal, because synapsis is never successfully accomplished. Here again the chromatin alone is concerned.

As is well known, it is entirely possible, on the other hand, to produce 'intermediate hybrids.' But the study of cross-fertilized echinoderm eggs has shown that it is not the fusion of paternal and maternal plastochondria that produces these, but the retention of paternal chromatin by the fertilized egg. This always occurs, apparently, in forms sufficiently closely related.

c. Normal fertilization in Nereis. No clearer proof of the secondary importance of cytoplasmic structures in fertilization and inheritance could be desired, however, than that furnished by the observations of the normal fertilization of the egg of *Nereis*, made recently by F. R. Lillie. One of the enigmas in the study of the cytology of the sex cells has been for many years the nature and function of the middle-piece. The fact that it alone accompanies the 'head' or nucleus of the spermatozoon into the egg in fertilization has been generally accepted as proof that it plays some essential part in that process. Indeed, it was the discovery that the middle-piece and its accessory structures are com-

posed of 'mitochondria' that led Benda, Duesberg and others to claim for these bodies a continuity and significance in heredity quite equal to those of the chromosomes.

But Lillie finds that the middle-piece never enters the egg in Nereis. This discovery, therefore, renders this interpretation of the importance of 'mitochondria' exceedingly doubtful. On page 427 this author discusses this question as follows:

The only characteristic thing about the cytoplasmic elements introduced by the spermatozoon is their great variability as to quantity and character in different animals. In *Ascaris* a very large quantity of cytoplasm containing characteristic plastosomes is introduced, as Meves has shown. In many, probably most, forms with flagellated spermatozoa, the entire spermatozoon enters; in some echinids the tail is left without, and in *Nereis* both tail and middle-piece fail to enter; and turning to plants, in phanerogams, apparently nothing but the nucleus is eventually concerned. There is nothing on the cytoplasmic side to correspond with the regularity of the nuclear phenomena in both animals and plants. In such precise phenomena as those of inheritance a mechanism of equal precision is to be expected, and it must be admitted that on the cytoplasmic side no such mechanism has been discovered. Moreover, as the laws of inheritance are the same for both animals and plants, a similar mechanism must exist for both, and such has been discovered only in the nuclei of the gametes. There is bad logic in the assumption that whatever parts of the spermatozoon enter the egg are necessarily concerned in the mechanism of transmission in inheritance, and the view that the cytoplasmic elements of the male gamete are concerned primarily in accessory functions of fertilization, such as locomotion and penetration, is still well founded.

The nature and function of 'mitochondria' or plastochondria

I believe the cases just cited argue strongly against Meves' interpretation of the importance of the plastochondria in *Ascaris*, and of the 'mitochondria' in other forms. If these plastin granules are of such great importance in heredity as Meves, Benda, Duesberg and others believe, it is difficult to understand why such a considerable proportion of them should be lost by the sperm during the course of its development. In *Nereis* we see the loss of this material just before fertilization occurs; but in many forms it takes place earlier. It has already been stated that a reduction in the amount of cytoplasm occurs in the young

spermatids of *Ascaris* by which the size of these cells is greatly lessened. Many plastin granules are lost when this cytoplasmic lobe is thrown off (fig. 13). Such a reduction in the spermatid with the consequent loss of these inclusions has been described by Struckmann in *Strongylus*, by Meves in the guinea-pig, by van Korff in *Phalangista*, by Broman in *Myxine*, by von Ebner in *Rana* and in certain mammals where he refers to these granules, probably, when he speaks of 'tingierbare Körner.' Duesberg observed it in the rat and Jordan in the opossum, while Rosenberg reports its occurrence in the *Arachnida* and Vejdovsky in *Turbellaria*. Faure-Fremiet reports the loss of all of these granules in the cytoplasmic lobe thrown off by the spermatid of *Arion*. Professor Montgomery discovered a remarkable case of the loss of 'mitochondria' in *Peripatus*. Just before the transformation of the spermatid into the spermatozoon all the mitochondrial material aggregates into a fairly compact body, and this is always included in the cytoplasmic lobe which is thrown off, so that the mature sperm never contains any of it. If, then, this material is of such great significance in heredity, it must be explained why it is so generally partially or wholly lost by the developing spermatozoon, in forms ranging all the way from the *Turbellaria* to mammals.

The true origin of 'organ-forming substances'

I cannot close this brief discussion of the significance of cytoplasmic inclusions in germ and body cells without referring to the work of Conklin on ascidian eggs and larvae. No one has traced the heredity of specific larval tissues from definite cytoplasmic inclusions more accurately and continuously than he. In the fertilized but unsegmented egg of various ascidians, notably *Cynthia*, Conklin found a constant distribution of pigment in the cytoplasm. This marks a definite localization of masses of protoplasm. These masses differ potentially from one another, as Conklin proved by the fact that he could trace the formation or origin of the various germ layers, tissues and organs of the larva as cleavage progressed to one or another of these masses,

since the identity of each was made certain by its specific pigmentation. But the localization and pigmentation of these masses of cytoplasm—'organ-forming substances,' as Driesch calls them—is not complete until after the break-down of the nuclear membrane. Indeed, the material which forms the 'clear zone,' from which arise the ectoderm and its derivatives, actually comes out of the nucleus. All the other masses also are derived as certainly from the nucleus, though not so directly, according to Conklin's observations. After describing the formation of the clear zone in gasteropod and Ascidian eggs, he says (page 101):

This truly remarkable condition in which considerable portions of the cytoplasm are traceable to the nucleus is of the utmost theoretical importance. From all sides the evidence has been accumulating that the chromosomes are the seat of inheritance material, until now this theory practically amounts to a demonstration. On the other hand, all students of the early history of the egg have observed that the earliest visible differentiations occur in the cytoplasm, and that the position, size and quality of the cleavage cells and of various organ bases are controlled by the cytoplasm. However, in the escape of large quantities of nuclear material into the cell body and the formation there of specific protoplasmic substances we have a possible mechanism for the nuclear control of the cytoplasm; and when, as in the case of the ascidians and fresh water gasteropods, these substances are definitely localized in the egg, and can be traced throughout the development until they enter into the formation of particular portions of the embryo, a specific mechanism for the nuclear control of development is at hand, and the manner of harmonizing the facts of cytoplasmic organization with the nuclear inheritance theory is clearly indicated.

SUMMARY

1. Cytoplasmic inclusions of whatever kind found throughout the course of spermatogenesis of *Ascaris megalocephala* are reducible to two materials, both of which are of nuclear origin: (a) the karyochondria, which are derived directly from the karyochromatin, and (b) the plastochondria, which are derived from the plastosome, in part, and in part from the karyochondria.

2. Both of these appear first in the nucleus of the spermatogonium. The karyochondria form the surface layer of the karyochromatin, whether this is in the form of chromosomes or not. The plastochondria form the plastosome.

3. In the young spermatocyte the karyochondria pass through the nuclear membrane into the cytoplasm, where they form the 'refringent granules.' These at once begin the elaboration or secretion of yolk within themselves, and so become the 'refringent vesicles.' These then fuse to form the 'refractive body,' as the spermatid transforms into the spermatozoon. This transformation takes place entirely within the vas deferens.

4. The refractive body is purely a food supply for the use of the spermatozoon, and frequently it is entirely consumed before the spermatozoon enters the egg. It therefore plays no part in fertilization; hence, the only function of the karyochondria in *Ascaris* is to form yolk.

5. The plastochondria are, like the plastosome, merely residua, and show negative behavior wherever they occur. While many are retained by the spermatozoon, many are lost by the spermatid.

6. Through the use of a single basic or nuclear stain, these two kinds of cytoplasmic inclusions have been confused. The simplest 'double stains' distinguish them clearly.

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All figures were drawn with the camera lucida, on the table level, with one-twelfth oil immersion objective and ocular 18, except figures 15, 16, 18 and 39 to 48 inclusive, which were drawn with ocular 4. Figures 41 to 48 inclusive have not been reduced. All others have been reduced one-third.

PLATE 1

EXPLANATION OF FIGURES

Figures 1 to 18 inclusive represent cells fixed in Flemming's strong solution, and stained according to Benda's method, modified as stated on page 426. In all of these, the basi- or karyochromatin stains red-brown, the oxy- or plastrochromatin and its derivatives, the plastrochondria, yellow, and the karyochondria dark blue.

1, 2, 3 Spermatogonia of different ages.

4, 5, 6, 7, 8, 10, 11 Developing spermatocytes, which show the escape of the karyochondria from the nucleus into the cytoplasm, and the formation of yolk within them. Also the escape of the plastrochondria from the nucleus into the cytoplasm, and the great increase in the relative amount of the cytoplasm.

12 A fully formed spermatid. The mitochondria of Mayer or plastrochondria of Meves are distributed radially around the chromatic mass throughout the 'perinuclear zone.'

14 A spermatid undergoing cytoplasmic reduction.

15 A longitudinal section of a fully formed spermatozoon.

17 A spermatozoon in the 'entrance region' of the uterus, showing no trace of the refractive body.

18 An egg in the metaphase of first cleavage. The chromosomes are not cut, so that the karyochromatin is entirely hidden by the karyochondria. The astral rays, centrosome, etc. are distinctly yellow.

19 to 27 Cells fixed in the Carnoy-Lebrun acetic fluid, and stained in the Ehrlich-Biondi stain. In these the karyochromatin is green, the plastrochromatin and plastrochondria red, and the yolk vesicles purplish (not so dark as in the colored figures). The karyochondria are not to be distinguished from the karyochromatin in cells stained in Ehrlich-Biondi.

25 Fusion of the yolk vesicles preparatory to the formation of the refractive body in the vas deferens.

27 A spermatozoon in the 'entrance region' of the uterus which has almost entirely consumed its food supply (the refractive body) during its journey.

28 Here the food supply is entirely gone. This cell was fixed in acetic-alcohol, a modification of Zur Strassen's fluid, and stained in iron-hematoxylin and Bordeaux red. The plastrochondria take the latter.

29 A spermatogonium fixed in acetic-alcohol and stained in iron hematoxylin alone. The plastrochondria (plastosome) here stain jet black.



1



8



13



23

PLATE 2

EXPLANATION OF FIGURES

30-40 All figures from cells fixed in acetic-alcohol.

30, 31 Older spermatogonia, stained in iron hematoxylin alone.

32 Young spermatocyte stained in iron hematoxylin and Bordeaux red. Karyochromatin blue-black, oxychromatin or plastochondria red and yolk vesicles blue to light blue.

33, 34 Maturation divisions of spermatocytes, stained in iron hematoxylin alone, and very heavily destained. The mitochondria of Mayer, Romieu, Faure-Fremiet, and others, form axial chains in the fully formed yolk vesicles.

35, 36, 37, 38 Various stages in the formation of the refractive body in the vas deferens.

39, 40 Eggs being entered by spermatozoa, which show various stages in the consumption of the refractive body.

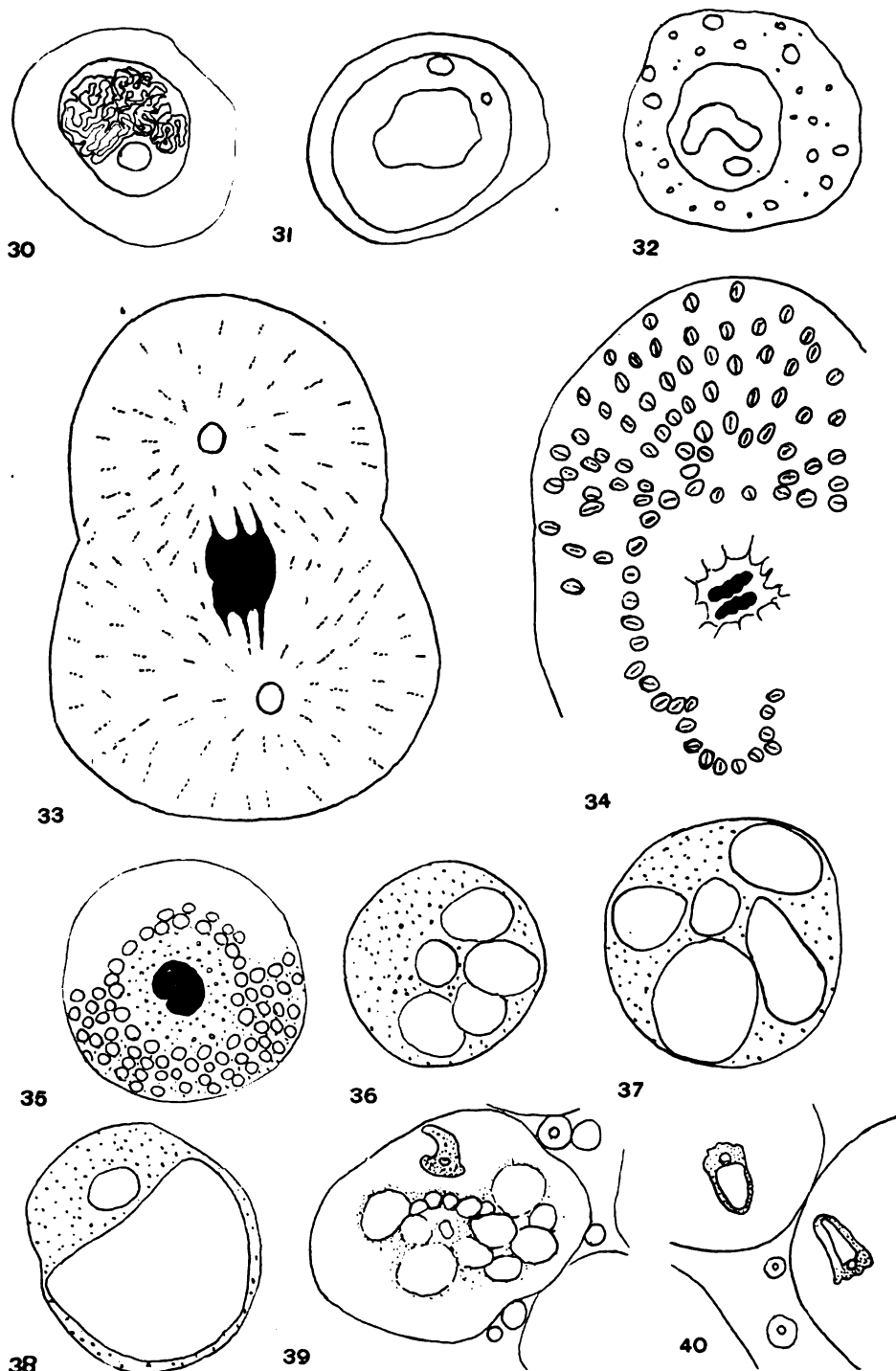
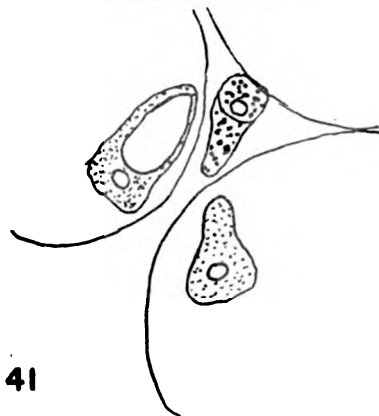


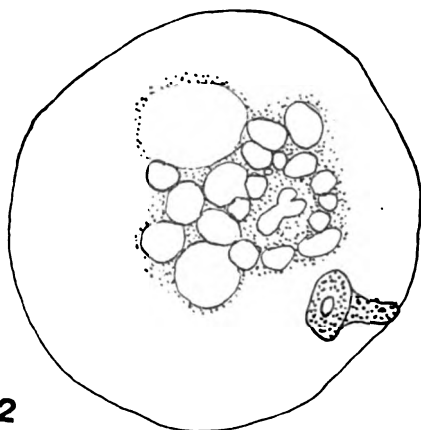
PLATE 3

EXPLANATION OF FIGURES

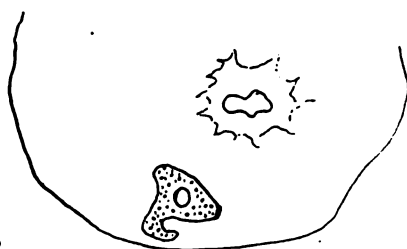
- 41-48 All figures from cells fixed in acetic-alcohol and stained in iron hematoxylin and Bordeaux red.
- 41, 42, 47 Sectioned eggs.
- 43, 44, 45, 46 Whole eggs shown in optical section.
- 48 A mature spermatozoon with refractive body intact.



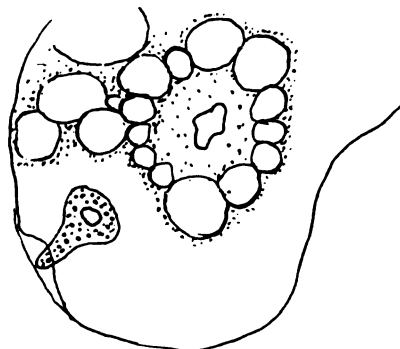
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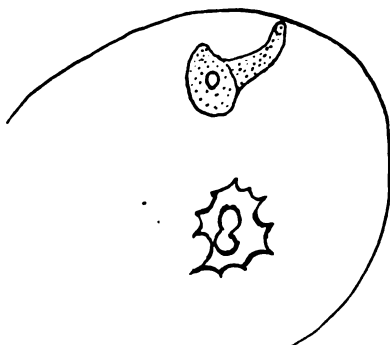
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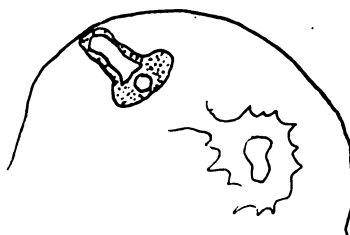
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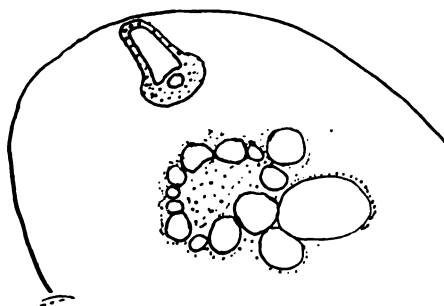
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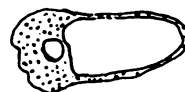
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